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Carcinogenesis with Ultraviolet Radiation of Wave Length 2,800–3,400 Å*

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INTRODUCTION

Because of the excellence of ultraviolet radiation as a carcinogenic agent the attention of several investigators has recently been directed toward the determination of the active wave lengths involved and the amount of energy necessary for the process (6, 12, 14, 16). Experiments in which mice were irradiated with the entire spectrum of the mercury arc, through various filters, and with other sources of known spectral distribution indicated that the region 2,800–3,400 Å was most active (16). While this conclusion seemed to be in harmony with the results of other investigators (2) it appeared desirable, nevertheless, to demonstrate the carcinogenicity of these wave lengths in the absence of other parts of the spectrum. Also, a determination of the energy necessary for tumor production with this band would allow an investigation of the possibility that other wave lengths, while not essential for carcinogenesis, may have contributed to the process when the entire spectrum of the mercury arc was used. These aims have been accomplished through the use of a specially designed filter that transmits only those wave lengths between 2,800 Å and 3,600 Å and is of sufficient size to permit the simultaneous irradiation of a considerable number of mice.

EXPERIMENTAL

The general irradiation procedure was the same as has been described previously (16). Young adult white ABC mice of both sexes were used. They were kept on shavings in ordinary metal box cages, with Purina dog chow and water available at all times except during the irradiation, when they were placed in a special cage 25.5 cm. square and 3 cm. deep, constructed of wire mesh and divided into 24 individual compartments to prevent the mice from huddling together and to minimize movements (16). In all cases the mice were irradiated 30 minutes a day, 6 days a week, with the light of a medium pressure mercury vapor lamp [1] passed through a special filter. The irradiation was continued until tumors appeared on the ears of more than 75 per cent of the mice in any given group. The intensity of the incident energy was adjusted by varying the distance from the mice to the lamp. Only one cage of mice was irradiated at a time, since the area covered by the field of the filter was not large enough to accommodate more.

The isolation of the spectral band between 2,800 Å and 3,400 Å without considerable loss of energy was not readily accomplished by means of glass filters alone. Therefore a combination liquid and glass filter was devised. In order that the transmittance of the filter might be as great as possible within the isolated range, the number of interfaces was kept at a minimum by using the glass filters to enclose the liquid. The filter cell (Fig. 1) was constructed by cementing 4 strips of pyrex glass $16.5 \times 1.8 \times 0.5$ cm. together at the ends, forming a square. The strips were ground on the edges and ends to form true, flat surfaces for joining. The cement used was glyptal No. 1202,[2] which was heated to 140° C. for 1 hour to harden. One face of the cell was a flat, standard, polished sheet of Corning corex No. 970 glass. This was cemented directly to the square of pyrex strips, forming a shallow dish 1.8 cm. deep and 16.5 cm. square. The other face of the cell was a polished sheet of Corning No. 986 special filter glass approximately 3 mm. thick. This was attached to the pyrex square by means of a rubber gasket built up of a vulcanized latex.[3] It was found

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[1] Manufactured by the Burdick Corporation, Milton, Wisconsin.

[2] Obtained from the General Electric Company, Schenectady, N. Y.

[3] Obtained from the Vultex Chemical Company, Cambridge, Massachusetts.

that a gasket cut from light, extremely elastic sheet rubber [4] was as good as the one made from latex. This gasket was necessary to provide an elastic component to compensate for the difference in expansion between the No. 986 glass and the rest of the cell.

Three holes were drilled in one of the pyrex strips to provide an inlet for filling the cell and an inlet and outlet for cold tap water, which was circulated through Both cooling coil and inlet tube, of 4 mm. pyrex tubing, were provided with gaskets of rubber tubing at their point of passage through the side of the cell. The inlet tube was connected with a small leveling bulb to take up the expansion of the filter liquid, which was a 1.3 *N* aqueous solution of nickel sulfate (8).

The whole glass assembly was contained in a wooden

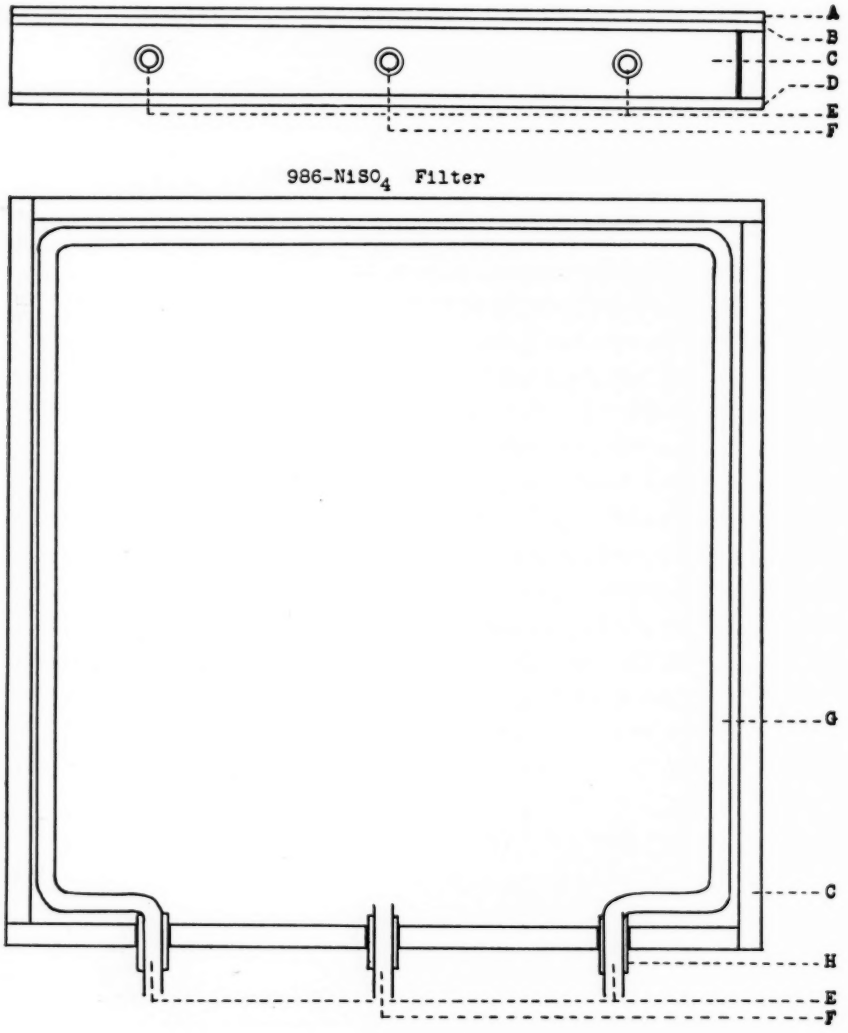

FIG. 1.—A = Corning filter: red-purple corex A No. 986, polished to standard thickness. B = Rubber gasket. C = Pyrex strips. D = Corning heat-resisting clear corex D glass No. 970, polished to standard thickness. E = Inlets for cooling coils. F = Inlet for leveling bulb and filling. G = 4 mm. pyrex glass tubing cooling coil (two turns). H = Rubber tubing packing.

a cooling coil around the inside edges of the cell. The coil was made of two turns of 4 mm. pyrex glass tubing bent in a square and inserted before final assembly. This coil was found necessary to prevent undue expansion of the cell and actual boiling of the filter liquid from the heat of the lamp. Also, the No. 986 glass was found to be rather sensitive to heat and it was advisable to protect it as much as possible.

[4] Obtained from the Goodrich Rubber Company, Akron, Ohio.

holder, which, in turn, was attached to a metal shield. The filter and shield were suspended beneath the medium pressure mercury arc with the No. 970 corex glass nearest the arc, and in such a manner that the mice were protected from all radiations except those passing through the filter.

The transmission of the filter was determined by taking spectrograms (Fig. 2) through it in the region 2,700–3,800 Å. A large Bausch & Lomb quartz spec-

trograph was used with an H-4 General Electric mercury lamp source whose glass envelope had been pierced to allow the ultraviolet lines to reach the slit. Also, a transmission curve was made with a Bausch & Lomb quartz monochromator and a clinical quartz mercury arc as a source, and a sensitive vacuum thermocouple in conjunction with a type LS Leeds & Northrup galvanometer as an energy-measuring instrument. Less than 2 per cent of the light at 3,650 Å was transmitted, transmission at 2,803 Å was zero, while at 3,131 Å it was approximately 40 per cent. Thus it was demonstrated that the filter transmitted only those wave lengths of the mercury arc lying between 2,800 Å and 3,600 Å. Since there are no lines in the mercury arc spectrum between 3,341 Å and 3,650 Å this means essentially that only energy lying within the range 2,800–3,400 Å was used in this study.

The energy incident upon the irradiation cage was measured with a multiple junction copper-constantan thermopile together with a type LS galvanometer. The combination had a sensitivity of 19.3 ergs/cm.^{2}/mm. deflection. The amount of energy received by the irradiated animals was determined by averaging a group of readings taken at various points on the irradiated surface.

The mice were irradiated in three groups, each group being given a different intensity of radiation. The intensities were 2,100, 4,200, and 7,500 ergs/cm.^{2}/second, resulting in daily doses of 0.38, 0.76, and 1.35×10^7 ergs/cm.^{2} respectively. The results were expressed as the number of days necessary for the appearance of tumors in 50 per cent of the animals within any one group (4, 16).

RESULTS AND DISCUSSION

When the mice were exposed to the entire spectrum of the mercury arc (5, 16) it was found that a pronounced erythema and thickening of the ears resulted, as well as considerable irritation and tissue destruction. Scratching of the ears by the mice, which further aggravated the tissue damage, was a usual accompaniment of irradiation. In addition to these local effects, a generalized toxicity appeared at the higher energy levels of irradiation and the mice became more susceptible to infection. When the filter was used, however, the erythema, scratching, thickening of the ears, and tissue destruction were much less evident. The animals remained in good health and exhibited no toxic symptoms attributable to the irradiation. This indicated that the deleterious effects observed during irradiation with the entire mercury arc were probably due to the action of high total dosage and not to the carcinogenic portion of the spectrum *per se*.

In every group more than 75 per cent of the mice

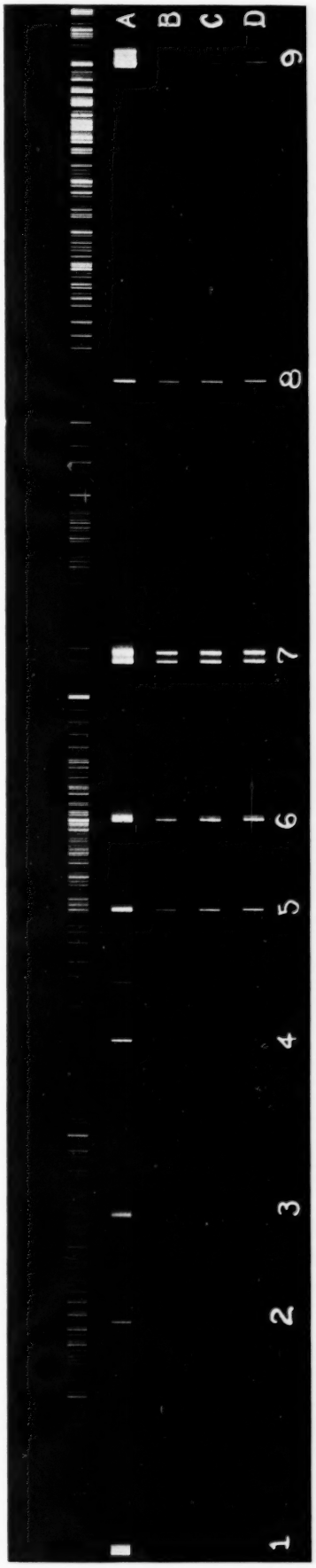

FIG. 2.—Spectrograms of the mercury arc taken on a Bausch & Lomb large quartz spectrograph, position IV, with and without the interposition of the filter. The top line is an iron reference spectrum. Line A is the mercury arc without the filter, exposed 2 seconds. Lines B, C, and D are the mercury arc with the filter, exposed 2, 5, and 10 seconds respectively. The rows are the principal lines of the mercury arc as follows: (1) 2,652 Å, (2) 2,752 Å, (3) 2,803 Å, (4) 2,893 Å, (5) 2,967 Å, (6) 3,021 Å, (7) 3,131 Å, (8) 3,341 Å, (9) 3,650 Å.

ultimately developed ear tumors. Generally the irradiation was discontinued when this incidence was reached, since previous experience had shown that valid comparison could be made on the basis of the time necessary to develop a tumor incidence of 50 per cent within a group (4, 16). With daily doses ranging from 0.38 to 1.35×10^7 ergs/cm.^{2} the number of days for 50 per cent of the mice to develop tumors ranged from 362 to 196, and the total amount of energy applied ranged from 116 to 212×10^7 ergs/cm.^{2} (Table I). This demonstrated that carcinogenesis could be effected with wave lengths 2,800–3,400 Å exclusively.

Previous work had shown that within limits the intensity of radiation bears no relation to the rate of tumor formation, but that the daily dosage correlates with the rate (5, 6). A study of the data in Table I bears out this point, namely, that the time necessary to develop 50 per cent tumors increased definitely with decreasing daily dosage. Blum, Grady, and Kirby-Smith (5) found that with dosages above 10^8 ergs/cm.^{2}/week there was no significant change in time necessary to develop a tumor incidence of 50 per cent, but that below this value time varied inversely with dosage. All the experiments reported in the present study involved dosages well below this maximum, and to that extent our data confirm the conclusion of Blum and his associates. In other words, essentially the same results were obtained with a restricted band of light as with the whole spectrum.

TABLE I: THE RELATION BETWEEN DOSE AND RATE OF TUMOR PRODUCTION WITH ULTRAVIOLET LIGHT OF WAVE LENGTH 2,800–3,400 Å

Group	Effective total *	Daily dose, ergs/cm.^{2}	Length of time to 50% tumors, days	Total energy, ergs/cm.^{2}
I	16	0.38×10^7	362	116×10^7
II	16	0.76	256	166
	21	0.76	237	152
III	26	1.35	218	207
	13	1.35	196	212

* Number of animals surviving at time of appearance of first tumor.

The data in Table I indicate also that the total energy necessary for the development of tumors varied directly with the daily dose of radiation. A small amount of energy applied every day over a long period of time was more efficient from the energy standpoint than a larger daily dose applied over a shorter time. These data indicate that there may have been a waste of energy when higher doses were employed, or that high energy levels could actually have been toxic to the cells, thus retarding carcinogenesis. Essentially the same conclusion had been reached by Cramer and Stowell (10) when methylcholanthrene was used as the carcinogenic agent. It is interesting to note that there was also a similarity between the distribution of dose-response relationships of the two agents with regard to time (5, 9). These phenomena may indicate that some fundamental disturbance results during carcinogenesis common to both ultraviolet radiation and the hydrocarbons, even though no additive effect between these two carcinogens has been observed (15, 17).

Fig. 3 compares the results obtained in this study with older results in which the whole spectrum of the mercury arc was utilized. The number of animals used in the present experiment was too small to permit exact analysis, but rough comparisons seem allowable. Such comparisons were facilitated by the preparation of curves obtained by plotting the log of "Days to 50 per cent tumors" against total dose. While straight lines were obtained, too much significance should not be ascribed to the shape of the curve since the data used were restricted within certain limits. Curves B and C represent the results obtained when no filter was used, B taken from the studies of Rusch, Kline, and Baumann (16) and C from the data of Blum, Grady, and Kirby-Smith (5). The results of the latter investigators have been corrected to give only that energy lying within the band 2,800–3,400 Å. Such corrections are necessary in order that a comparison can be drawn between the results

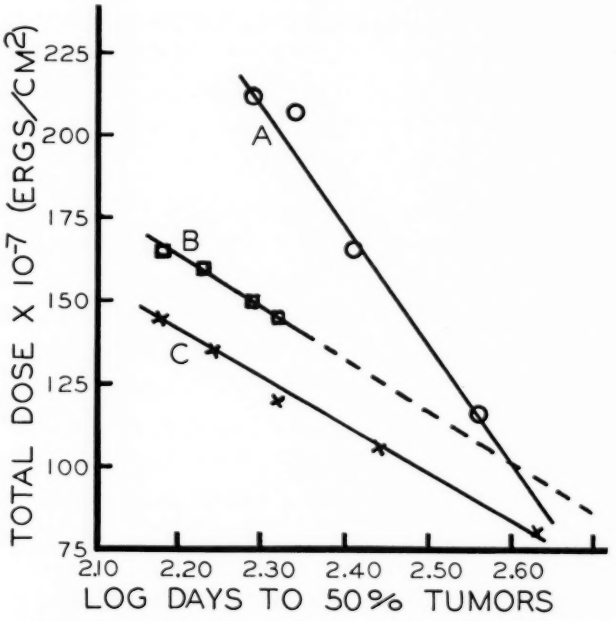

FIG. 3.—Curve A plotted from data obtained in the present study with a filter to isolate the wave length band 2,800–3,400 Å. Curves B and C plotted from data obtained by Rusch, Kline, and Baumann (16) and Blum, Grady, and Kirby-Smith (5) with the whole mercury arc spectrum but calculated to the basis of the energy lying within the range 2,800–3,400 Å (see text) for comparative purposes.

of the two investigations. These corrections were made by estimation from the spectral distribution of the arc (6), and the corrected energy values are the product of a factor that is the sum of the intensities of the lines within the region 2,800–3,400 Å, over the sum of the intensities of all the lines within the region measured by the photocell of these investigators, times any given dose. Inspection of the curves shows that the two quantities "Days to 50 per cent tumors," and dose, bear an inverse relationship to each other in all cases. The results of Rusch, Kline, and Baumann and those of Blum, Grady, and Kirby-Smith, both of which were obtained with the whole mercury arc spectrum, agree within limits of experimental error, and at the lower energy levels those obtained in this study (curve A) agree with the other two. However, at high energy levels the light passed through the filter seemed to be relatively less efficient in the production of tumors. One possible explanation for this discrepancy is that wave lengths other than those in the band 2,800–3,400 Å may be either carcinogenic or cocarcinogenic. Another possibility is that the mice were subjected to a considerable amount of heat (infrared) when no filter was used, and this may have been a factor in the carcinogenic process. Since at higher energy levels the heat was disproportionately increased, this might explain the difference between the slope of curve A and the slopes of curves B and C. Still another possibility is that when the whole spectrum was used at higher energy levels the ears of the mice were burned, considerable tissue destruction resulted, and the irritation or resultant attempts at repair may have acted in a cocarcinogenic way (1, 11, 13).

Recently Blum and Lippincott (7) have reported the production of tumors with 2,537 Å exclusively. One tumor was noted also by Rusch, Kline, and Baumann (16) in mice irradiated with a cold quartz lamp that produces 89 per cent of its output at 2,537 Å. However, the latter investigators attributed this tumor to the presence of sufficient energy in wave lengths known to be carcinogenic. In both investigations it was found necessary to use exceedingly high dosages and to irradiate for long periods. If the same amount of energy of the wave lengths 2,800-3,400 Å had been employed the tumor incidence would have been at least 50 per cent and probably more. The screening effect of the outer layers of the skin was suggested as largely responsible for the weak carcinogenic effect of 2,537 Å, and transmission data were presented that supported this point (7). In the ordinary mercury arc wave lengths below 2,800 Å constitute about 35 per cent of the energy in the region 2,000-3,400 Å, and hence the difference between filter and nonfilter experiments might be attributed to this fraction of the total spectrum. However, of these wave lengths only 2 per cent is transmitted through the outer layers of the skin to the basal cells. Thus while shorter wave lengths apparently are intrinsically carcinogenic, it is not probable that wave lengths lying below 2,800 Å contribute importantly in short term experiments. It would seem, therefore, that for practical purposes the wave lengths 2,800–3,400 Å may be considered as those responsible for tumor formation. Nevertheless, the information that under proper conditions other wave lengths are carcinogenic is of real importance in dealing with questions of the mechanism of tumor formation.

While the mode of action of ultraviolet light in the production of cancer is still obscure, the suggestion (3, 17) that some disturbance in the proteins or nucleic acids of the cell might be responsible merits further consideration. This idea receives strong support from the fact that the absorption maxima of these particular cellular constituents correspond closely with the carcinogenic portion of the spectrum. It is to be expected, therefore, that various disturbances in these components might result from ultraviolet irradiation and it seems likely that such disturbances would have a profound effect upon cellular behavior.

SUMMARY

A filter has been constructed for the isolation of the wave length band 2,800–3,400 Å with the least possible loss of energy. Tumors were produced in mice by exposure to the wave length band 2,800–3,400 Å. Carcinogenesis could be effected but more energy of these wave lengths was needed than when the whole mercury arc spectrum was employed, an implication that certain other portions of the spectrum can influence carcinogenesis. Small amounts of energy applied over a long period of time appeared to be more efficient for tumor production than large doses given during shorter periods. This indicated that there was a waste of energy when large doses were employed, or that the higher levels had an additional retarding influence.

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The Effect of Methylcholanthrene upon Epidermal Sodium and Calcium*

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Previous studies have shown that treatment of the epidermis of mice with 0.6 per cent methylcholanthrene in benzene brings about a notable decrease in total iron (3) and in total lipid (5). It was thought desirable, under similar conditions, to ascertain the influence of this carcinogen on epidermal sodium and calcium.

EXPERIMENTAL PROCEDURE

New Buffalo mice of both sexes 3 to 4 months old were used, as there was no apparent sex difference. The methods of shaving, of applying the carcinogen, and of separating the epidermis from the dermis have already been described (3, 1). As in the previous work with iron and ascorbic acid, nucleoprotein phosphorus was used as a basis of reference.

The total sodium content of the epidermis was determined by an ultramicromethod devised in this laboratory (2). For calcium the method (on a micro scale) of Lindner and Kirk (4) was employed.

Fresh samples of isolated epidermis were weighed to the nearest 0.1 mgm. and divided into two portions. The larger portion was dry ashed in a silica crucible at 450° C. in a muffle furnace until all the organic matter had been destroyed. The total ash was used either for a single sodium or calcium determination, because only 1 to 2 mgm. of ash were obtained from the epidermis of 10 to 12 control or benzene-treated mice. The smaller portion of the sample was treated (3) for the determination of nucleoprotein phosphorus (N.P.P.). Expression of the results on a weight basis was not accurate because of hydration changes that accompanied separation of the epidermis from the dermis at 50° C. However, this disadvantage can be obviated by expressing the results by the ratios sodium/nucleoprotein phosphorus (Na/N.P.P.) and calcium/nucleoprotein phosphorus (Ca/N.P.P.), since the samples were accurately weighed when divided.

* This investigation was aided by grants from The International Cancer Research Foundation and the National Cancer Institute.

RESULTS

Sodium

The effect of single and multiple applications of methylcholanthrene upon epidermal sodium is shown in Table I. The results are expressed as milligrams of sodium and milligrams of nucleoprotein phosphorus per 100 mgm. of epidermis, and by the significant ratio Na/N.P.P. The epidermis of normal untreated mice contained an average of 0.168 mgm. of sodium per 100 mgm. of tissue, and an Na/N.P.P. ratio of 1.17. The benzene-treated epidermis contained an average of 0.1626 mgm. of sodium per 100 mgm. of tissue, and an Na/N.P.P. ratio of 1.21. The application of methylcholanthrene, 1, 3, 6, 8, and 12 times over a period of 10, 10, 20, 20, and 30 days respectively, induced a small decrease in the total sodium content and in the ratios Na/N.P.P. However at 60 days, after 24 applications of the carcinogen, the ratio Na/N.P.P. of 1.18 was nearly the same as for the normal and benzene-treated epidermis. It is therefore concluded that the epidermal sodium content was not significantly affected by the application of methylcholanthrene.

Calcium

The influence of methylcholanthrene upon the epidermal calcium content is summarized in Table II. The results are expressed in the same manner as for sodium except that the ratio, Ca/N.P.P., was multiplied by 10. The average calcium content of normal and benzene-treated epidermis was 0.0435 mgm. and 0.0423 mgm. per 100 mgm. of tissue respectively. The ratio, Ca/N.P.P., of the former group was 3.60, that of the latter 3.40. After one application of methylcholanthrene the calcium content dropped to 0.0222 mgm. per 100 mgm. of epidermis and the ratio, Ca/N.P.P., to 1.58. This decrease was approximately 50 per cent of the normal. Multiple applications of the carcinogen, 3, 6, and 12 times for 10, 20, and 30 days respectively, caused a further slight diminution in the calcium content, and in the ratios, Ca/N.P.P. At 60

TABLE I: SODIUM/NUCLEOPROTEIN PHOSPHORUS RATIO OF MOUSE EPIDERMIS

Number of mice	Number of paintings	Time after first treatment to killing of mice, days	Na per 100 mgm. tissue, mgm.	N.P.P. per 100 mgm. tissue, mgm.	Na/N.P.P.
		NORMAL, UNTREATED MICE			
8			0.1707	0.135	1.26
7			0.1697	0.131	1.29
8			0.1490	0.149	1.00
8			0.1829	0.144	1.27
8			0.1677	0.164	1.02
39	(total) Average		0.1680	0.144	1.17
		BENZENE-TREATED MICE			
8	3	10	0.1684	0.116	1.45
9	3	10	0.1443	0.151	0.95
6	3	10	0.1661	0.134	1.24
6	3	10	0.1718	0.140	1.22
29	(total) Average		0.1626	0.135	1.21
		METHYLCHOLANTHRENE-TREATED MICE			
4	1	10	0.1471	0.132	1.11
6	1	10	0.1728	0.123	1.40
5	1	10	0.1585	0.131	1.21
15	(total) Average		0.1595	0.128	1.24
4	3	10	0.1390	0.117	1.18
5	3	10	0.1134	0.142	0.80
3	3	10	0.1971	0.135	1.46
5	3	10	0.1248	0.125	1.00
17	(total) Average		0.1435	0.129	1.11
5	8	20	0.1215	0.145	0.83
4	6	20	0.1227	0.140	0.87
4	6	20	0.1410	0.135	1.04
13	(total) Average		0.1284	0.140	0.91
6	1	19	0.1520	0.129	1.18
4	12	30	0.1269	0.127	1.00
5	12	30	0.1249	0.154	0.81
9	(total) Average		0.1259	0.140	0.90
5	24	60	0.1495	0.122	1.22
5	24	60	0.1413	0.130	1.09
5	24	60	0.1552	0.127	1.22
15	(total) Average		0.1486	0.126	1.18

TABLE II: CALCIUM/NUCLEOPROTEIN PHOSPHORUS RATIO OF MOUSE EPIDERMIS

Number of mice	Number of paintings	Time after first treatment to killing of mice, days	Ca per 100 mgm. tissue, mgm.	N.P.P. per 100 mgm. tissue, mgm.	Ca/N.P.P. ×10
		NORMAL, UNTREATED MICE			
7			0.0358	0.130	2.75
8			0.0420	0.117	3.60
11			0.0505	0.123	4.10
11			0.0400	0.105	3.80
10			0.0405	0.116	3.49
12			0.0520	0.116	4.48
59	(total) Average		0.0435	0.118	3.60
		BENZENE-TREATED MICE			
10	3	10	0.0495	0.124	4.00
12	3	10	0.0350	0.112	3.12
12	3	10	0.0348	0.133	2.55
10	3	10	0.0430	0.123	3.49
12	3	10	0.0467	0.129	3.62
9	3	10	0.0503	0.123	4.09
8	12	30	0.0380	0.130	2.92
73	(total) Average		0.0423	0.125	3.40
		METHYLCHOLANTHRENE-TREATED MICE			
6	1	10	0.0175	0.139	1.26
8	1	10	0.0273	0.142	1.92
6	1	10	0.0219	0.140	1.56
20	(total) Average		0.0222	0.140	1.58
7	3	10	0.0194	0.120	1.61
4	3	10	0.0199	0.107	1.86
5	3	10	0.0177	0.134	1.32
4	3	10	0.0192	0.138	1.39
20	(total) Average		0.0190	0.125	1.54
5	6	20	0.0166	0.122	1.36
5	6	20	0.0117	0.101	1.16
7	6	20	0.0235	0.131	1.80
7	6	20	0.0138	0.106	1.30
24	(total) Average		0.0164	0.115	1.40
5	12	30	0.0167	0.131	1.27
5	12	30	0.0121	0.137	0.88
7	12	30	0.0194	0.108	1.79
7	12	30	0.0140	0.108	1.29
24	(total) Average		0.0155	0.121	1.31
5	24	60	0.0216	0.161	1.34
6	24	60	0.0215	0.142	1.51
5	24	60	0.0228	0.152	1.50
16	(total) Average		0.0220	0.152	1.45

days, after 24 applications of methylcholanthrene, the epidermal calcium content of 0.0220 mgm. per 100 mgm. was nearly the same as that after one application of the carcinogen. The Ca/N.P.P. ratio of the epidermis treated 24 times with the carcinogen was slightly less than that of the epidermis receiving one application of the hydrocarbon.

The sodium/nucleoprotein phosphorus ratio–calcium/nucleoprotein phosphorus ratio is shown in Table III. The ratios for normal untreated and benzene-treated epidermis were 3.10 and 3.53 respectively.

TABLE III: SODIUM/NUCLEOPROTEIN PHOSPHORUS RATIO–CALCIUM/NUCLEOPROTEIN PHOSPHORUS RATIO OF MOUSE EPIDERMIS

Treatment	Number of paintings	Time after first treatment to killing of mice, days	$\frac{Na/N.P.P.}{Ca/N.P.P.\times 10}$
Normal		10	3.10
Benzene-treated		10	3.53
Methylcholanthrene-treated	1	10	7.56
" "	3	10	7.16
" "	6	20	6.50
" "	12	30	6.87
" "	24	60	8.13

After a single application of methylcholanthrene the ratio had increased to 7.56, and remained fairly constant to 60 days at which time the epidermis had received 24 applications of the carcinogen. The ratio for the methylcholanthrene-treated mice was 50 per cent greater than that of the normal and benzene-treated groups.

DISCUSSION

These chemical studies on the mode of action of methylcholanthrene on mouse epidermis have yielded several interesting facts. With a single application of methylcholanthrene the total amounts of iron and calcium were decreased to approximately 50 per cent of the normal. Moreover, multiple applications of the carcinogen on alternate days for 70 days caused a further lowering in total iron, while similar treatment for 60 days decreased only slightly the initial drop in total calcium induced by one application of methylcholanthrene. On the other hand, the ascorbic acid and sodium contents of mouse epidermis were not appreciably affected by treatment with the carcinogen.

It becomes apparent that the response of mouse epidermis to methylcholanthrene is very rapid and is chemically complex even in 10 day samples. It is therefore natural to assume that the process of epidermal carcinogenesis in mice cannot be ascribed to any one chemical change, but at least to several occurring simultaneously.

SUMMARY

One application of methylcholanthrene reduced the calcium content of mouse epidermis within a few days to approximately 50 per cent of the normal content. Multiple applications of the carcinogen on alternate days induced only slight further lowering. On the other hand, the epidermal sodium content was not significantly affected by similar treatment with the same carcinogen.

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The Occurrence and Transplantation of Embryonal Nephromas in the Rabbit*

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Embryonal nephromas or so called Wilms' tumors of the kidney have been found in a wide variety of animal species including man (2), swine (6), sheep (7), cows (8), rats (4), chickens (14), and rabbits. They are generally believed to occur with considerable frequency in the rabbit but a review of the literature yields only nine recorded cases (1, 5, 13, 15, 16, 17, 18), and this apparent low incidence is supported by the fact that observation of a large colony over a period of years has disclosed only four instances.

Investigation of these tumors in the lower animals has been limited largely to a morphological examination of autopsy material; clinical histories have not been available and transplantation has been uniformly unsuccessful. The growths observed in the present instance occurred in animals of known genetic constitution with complete life histories and, in addition, one was successfully transferred to normal rabbits. The object of the present paper is to record several features of the tumors that are at variance with the usual behavior noted in man and to describe the distinctive characteristics of the transplanted growth.

MATERIALS AND METHODS

The colony in which the neoplasms occurred was founded in 1929 for the study of constitutional problems (3) and its organization and management have been described in detail elsewhere (9). It was established at The Rockefeller Institute in New York City, moved to Princeton, N. J., in 1935, and since 1940 the majority of the animals have been housed at the Department of Pathology, Yale University School of Medicine, New Haven. The population is made up of fourteen pure breeds, including the Belgian, Beveren, Chinchilla, Dutch, English, Havana, Himalayan, Polish, Rex, Sable and Silver Marten, Siamese Sable, French Silver, and Tan breeds, and numerous hybrid lines. The colony is maintained in active breeding service, the pedigrees and life histories of all animals are known, and complete autopsies are performed after death.

The technic of anterior chamber transfer utilized in the transplantation experiments has been described (10) and the methods employed in testicular inoculations were similar to those in general use.

THE SPONTANEOUS TUMORS

Incidence.—The present report is based on 4 embryonal nephromas found in the colony from its inception in 1929 to January, 1943. More than 6,000 adult rabbits of both sexes came to autopsy during this 14 year period and the incidence of the tumors in the general population thus approximates 0.066 per cent.

The age, sex, and breed of the affected animals are shown in Table I. The recorded age refers to the time of death. All the animals were in good health and, with the exception of X6559, were killed without knowledge of the presence of the tumor. It is apparent therefore that the given ages are without significance as criteria of the time of tumor inception or the initiation of malignant properties. One of the affected animals was a male while the remaining 3 were females, but this apparent disproportion is inconclusive inasmuch as the female portion of the colony greatly outnumbered the male population. Tumor susceptibility also appears to bear no relationship to breed. It is of considerable interest, however, that 2 of the growths occurred in related Polish-Dutch hybrids. The sire of X6559 was also the grandsire of X20593-4 and their mothers were derived from the same cross. It is conceivable, therefore, that an hereditary factor independent of breed may be of significance in susceptibility to this form of neoplasia.

Clinical history.—No indication of the presence of a renal tumor was evidenced by the behavior of the animals during life. The females were normal, healthy, and vigorous and each bore several litters. However, a period of infertility distinguished the final 3 to 6 months of life in all cases. The male remained fertile throughout and was used extensively in breeding experiments until a severe infection with rabbit pox

* This investigation was aided by grants from The Jane Coffin Childs Memorial Fund for Cancer Research and from The International Cancer Research Foundation.

during the course of an epidemic necessitated disposal. The female ABl-3 was discarded for economic reasons following the termination of a series of genetic studies, while X20593-4 had been released from the breeding specifically to obtain fresh tumor for transplantation experiments.

Autopsy.—At autopsy alterations in the internal organs were found limited to the kidneys. Bilateral

TABLE I: OCCURRENCE OF EMBRYONAL NEPHROMAS IN A COLONY OF RABBITS OVER A FOURTEEN YEAR PERIOD

Animal No.	Breed	Sex	Age, months	Location of tumor	Date of discovery
498	Belgian	Male	27	Left	Jan. 23, 1933
AB1-3	American Blue	Female	23	Bilateral	Oct. 13, 1933
X6559	Dutch-Polish hybrid	Female	22	Bilateral	May 4, 1937
X20593-4	" " "	Female	17	Left	July 27, 1942

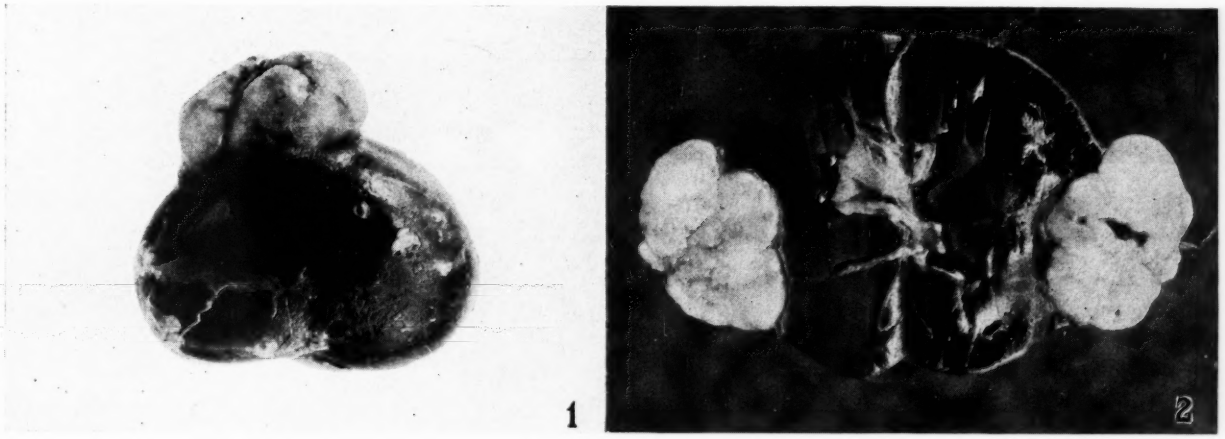


FIG. 1.—Kidney from rabbit 498. The left kidney alone was involved and the tumor occurred as a solitary mass. Mag. × 1.
FIG. 2.—Section through the kidney and tumor shown in Fig. 1. The tumor is sharply demarcated from the underlying renal parenchyma. Mag. × 1.

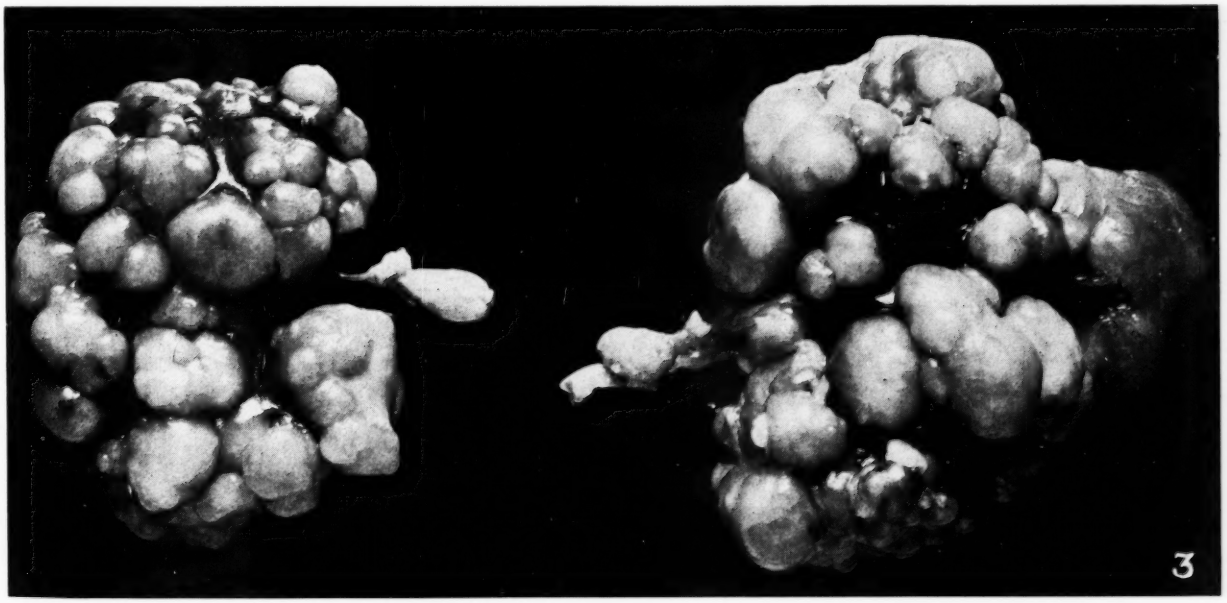
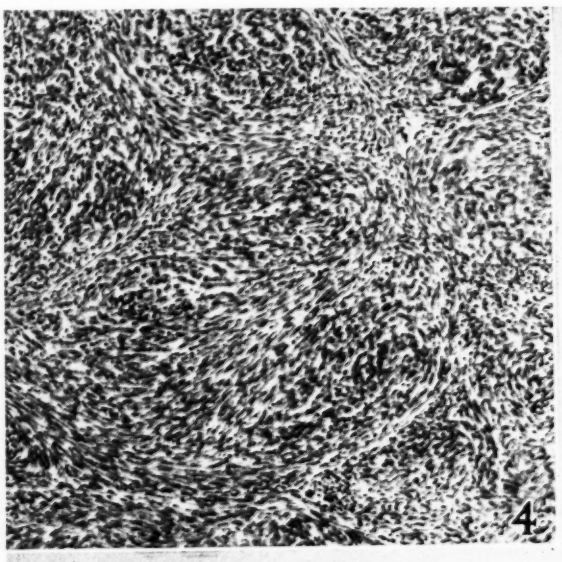
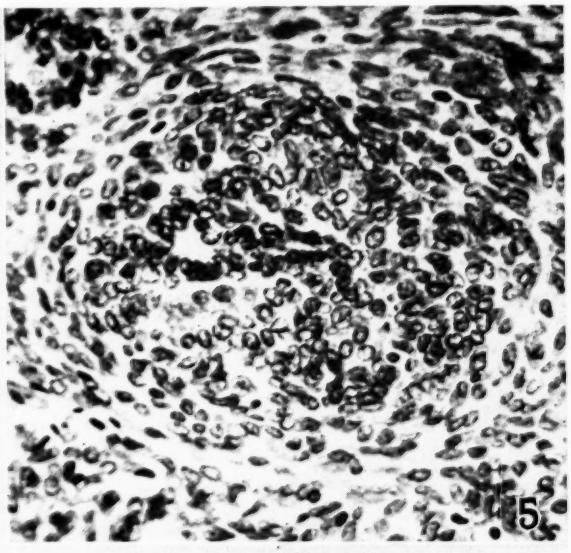


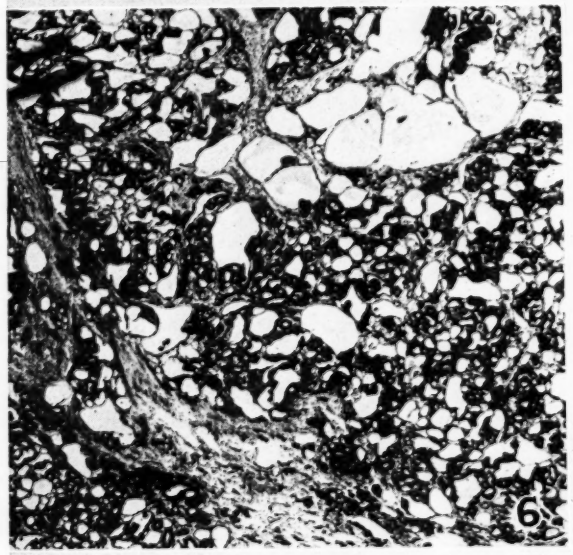
FIG. 3.—Kidneys from rabbit X6559. Multiple tumor nodules were scattered over the cortical surfaces of both kidneys. Mag. × 2.

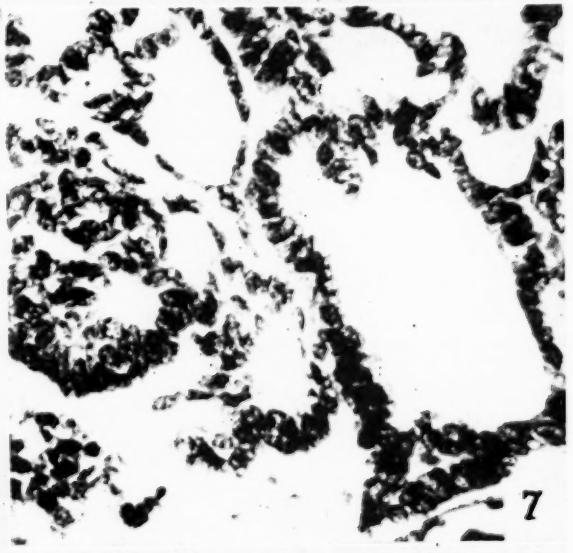
population for use in a routine tumor passage and was eventually killed to obtain tissue for serial transfer. On the other hand, the growth in X6559 was palpated during life and the animal was killed

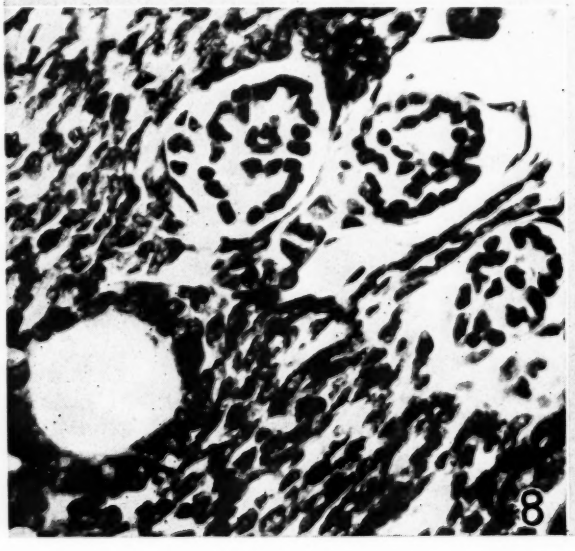
renal tumors were discovered in animals AB1-3 and X6559, while the left kidney alone was involved in rabbits 498 and X20593-4. The tumors were solitary in all animals with the exception of X6559, in which

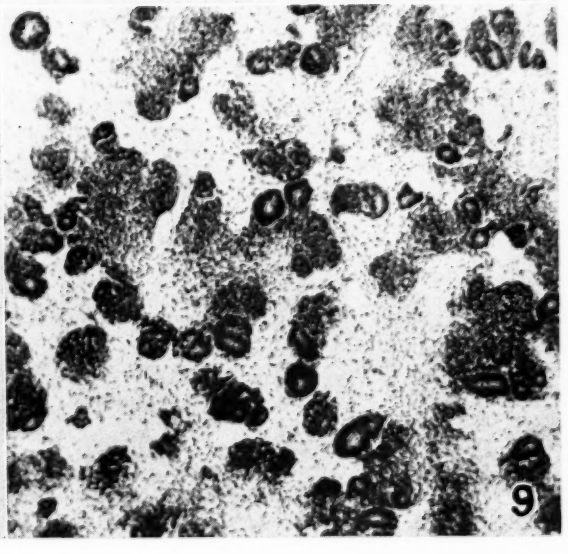












FIGS. 4-9

multiple larger and smaller, gray, fleshy nodules were scattered over the entire surface of the cortex (Figs. 1, 2, and 3). In all instances the neoplasms arose from the cortex and on section were well demarcated from underlying compressed renal parenchyma.

Histologically, the growths were distinguished by an embryonal structure identical with that found in analogous human tumors (Figs. 4 to 12). There were considerable architectural variations in different growths and in different parts of the same growth, but the essential elements were similar in all cases and consisted of cylindrical cells in adenomatous pattern and round or spindle cells in indifferent arrangement. Both elements were present in all the tumors but the proportion varied and appeared related to the degree of organization of the epithelial cells. Thus, in all the females epithelial cells were arranged in well defined tubules, and spindle cells, while numerous in some foci, were generally less conspicuous. In the male, on the other hand, the epithelial cells were grouped in solid clusters with only occasional evidence of alveolar formations and the surrounding intercommunicating bands of spindle cells made up the bulk of the tumor.

The tubular formations in the females varied greatly in size, and many appeared cystic. They were lined by single or multiple layers of epithelium that sometimes completely filled the lumen. Rarely, large foci of epithelial cells were encountered in which no attempt at organization could be found. Pseudoglomerular formations were common in the better differentiated areas.

Muscle, cartilage, or other tissues often seen in comparable human neoplasms were not found. Mitotic figures were rare except in the case of X6559. This case also differed from the others by the presence of islands of invading tumor cells in the renal parenchyma close to the parent growth.

Other organs and tissues of the body were thoroughly examined but neither gross nor microscopic metastases were found.

The Transplanted Tumor

Transplantation experiments were performed only in the case of animal X6559. Fragments of the tumor obtained immediately after death were transferred to the anterior chamber of the eyes of 9 adult rabbits. The rabbits were held under observation for a year but growth occurred in only one.

Growth was evident after 4 months in this case and the transplanted fragment had approximately doubled in size by the end of the eighth month. A biopsy was performed at this time and a portion of the tumor removed for serial transfer. The results of serial passage are shown in Table II.

Table II: The Results of Transplantation in the Anterior Chamber of the Eye and in the Testicle

Date	Generation	Number of rabbits	Number of takes
ANTERIOR CHAMBER SERIES			
Oct. 4, 1937	1	9	1
June 20, 1938	2	4	1
Dec. 21, 1938	3	8	6
May 25, 1939	4	7	2
TESTICLE SERIES			
Nov. 13, 1939	1	3	1
Oct. 17, 1940	2	6	2
Apr. 3, 1941	3	5	1
Aug. 14, 1941	4	5	1

One of the animals of the third anterior chamber generation was killed 11 months after transfer and fragments of the growth were implanted in the testicles of a series of males. A take occurred and the results of serial transplantation in this organ also are presented in Table II.

The most noteworthy characteristic of the transplanted tumor was its extremely slow growth rate. Takes were rarely evidenced in the eye before the 100th day, and in one instance growth persisted for 671 days without filling the chamber. A similar situa-

DISCRIPTION OF FIGURES 4 TO 9

All sections were stained with hematoxylin and eosin.

Fig. 4.—Section of kidney tumor from rabbit 498. The bulk of the tumor was made up of spindle cells arranged in wavy bands and enclosing small groups of round or slightly cuboidal epithelial cells. Mag. × 114.

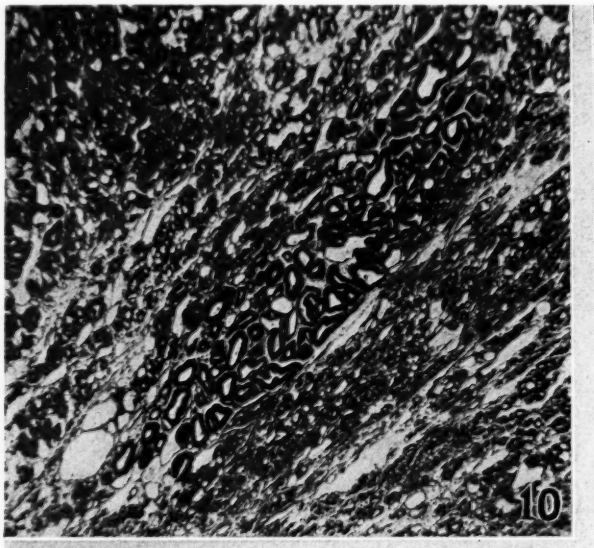
Fig. 5.—Higher power view of Fig. 4, showing a cluster of small round cells containing an alveolar structure. Mag. × 375.

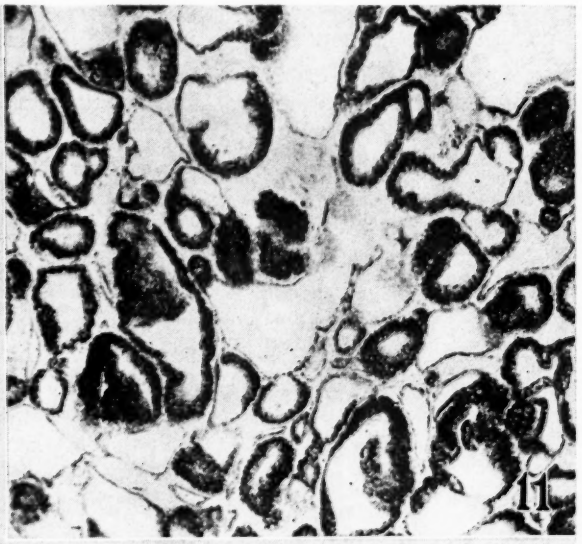
Fig. 6.—Section of kidney tumor from rabbit AB1-3. The greater part of this tumor was made up of epithelial elements with only scattered patches of spindle cells. The epithelial cells formed tubules of varying size and shape, many of which were widely dilated and cystic. Mag. × 26.

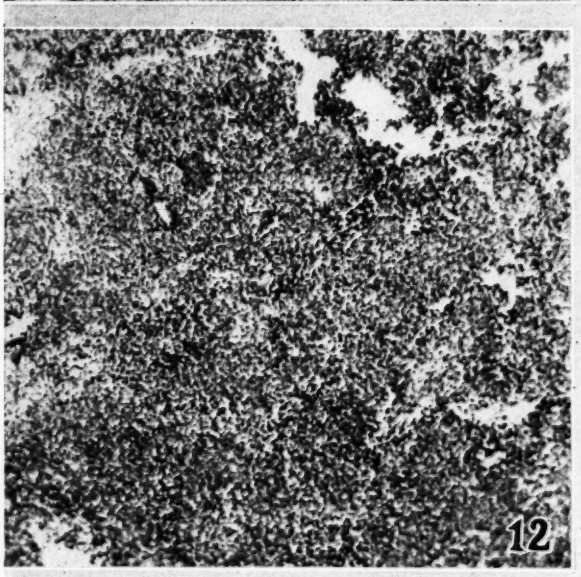
Fig. 7.—Higher power view of preceding section. Mag. × 475.

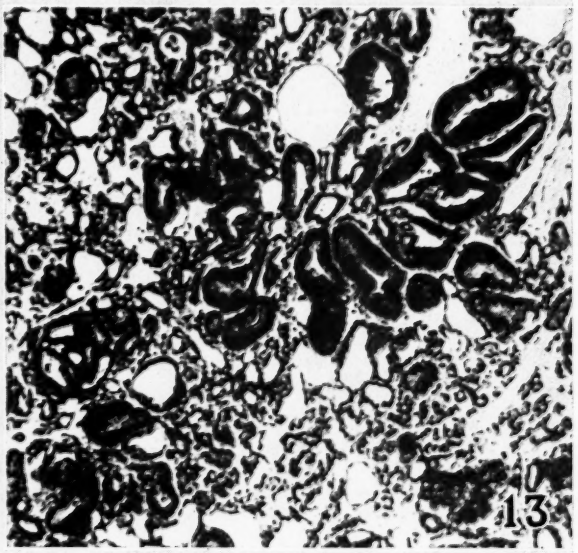
Fig. 8.—Section of tumor from rabbit AB1-3 showing a patch of spindle cells and 3 glomerulus-like structures. Pseudoglomeruli of this type were found in all the tumors under discussion. Mag. × 475.

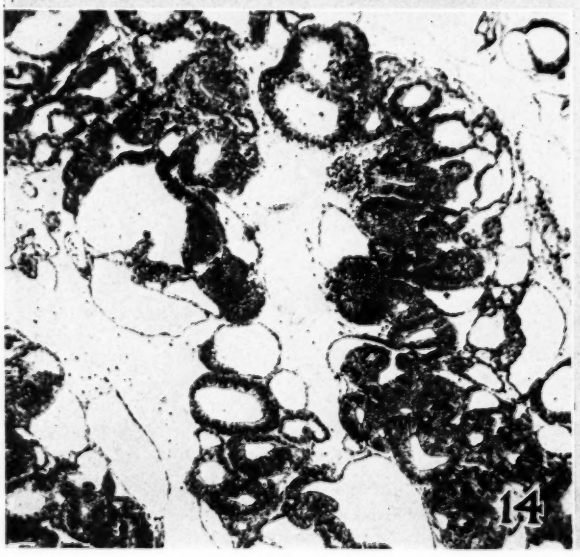
Fig. 9.—Section of tumor from rabbit X6559. This growth contained spindle cells, small round cells, and epithelial cells in approximately equal proportions. In many regions these elements were arranged as shown in the present figure, with well formed epithelial tubules and clusters of small round cells irregularly scattered in a loose matrix composed of spindle-shaped cells. Mag. × 85.

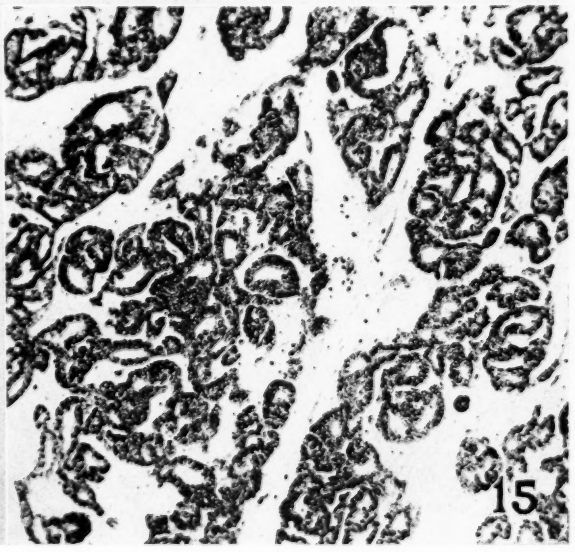












FIGS. 10-15

tion obtained in the testicle but, contrary to experience with other tumors, growth was generally more rapid in this organ and takes were often evident by the 60th day, with complete replacement of the parenchyma by the 300th day.

Despite an abundance of spindle cells in the fragment originally used for transfer, only the epithelial cells of the spontaneous tumor survived transplantation (Figs. 13 to 15). The arrangement of these cells was identical with that noted in the more adenomatous parts of the original growth and the histological picture was that of a well differentiated carcinoma. Mitotic figures were common and the structures of the eye and testicle were widely invaded, but extension beyond these organs or metastasis was never observed.

DISCUSSION

The incidence of embryonal nephromas in man is limited almost exclusively to childhood and the tumors are characterized by a rapidly fatal course. On the contrary, the tumors described in the rabbit were found in healthy adult animals, without lymphatic extension or metastasis and, with a single exception, were accidental autopsy findings. Moreover, transplantation studies involving the most advanced growth of the series gave additional evidence of their relative benignancy. Inasmuch as the tumors in the two species are morphologically identical, this extreme variation in biological behavior would appear to be a function of the host rather than of the tumor itself.

This suggestion is at odds with accepted practice in surgical pathology, where the assessment of the biological properties of a neoplasm is based solely on its morphological appearance and the constitution of the host is not considered a contributory factor. It should be emphasized, however, that in dealing with new growths of the human subject the interpretation of morphology rests on experience and observations confined to this species, and that while histology may reflect the behavior and potency of tumors in man the observed relationships do not necessarily carry over to other species (11, 12). The present tumors are a further example in point. Here morphology, interpreted on a basis of experience in man, indicates high malignancy, yet actual behavior, observed in both primary hosts and experimental animals, identifies the growths as the least malignant of all autonomous neoplasms in the rabbit.

The point also deserves some attention in its application to experimental cancer research. Experimental and spontaneous tumors in the lower animals are often referred to by terms identical with those used in human pathology, on the assumption that they represent analogous growths. Unfortunately, in the great majority of cases, this presumption is based on morphological similarity and a comparison of biological behavior is completely neglected. Thus appearance is stressed and character disregarded, and from the viewpoint of comparative tumor study the correspondence is often false and misleading. The tumors described in the present paper are morphologically identical with embryonal nephromas in man, but the wide variation in biological behavior precludes an analogy with this well defined clinical and pathological entity. It is essential, therefore, that results obtained from experimental studies be viewed in this light before they are applied to problems related to the human disorder.

SUMMARY

Observation of a large colony of rabbits over a period of fourteen years disclosed four instances of embryonal nephromas. Study of the growths in both spontaneous and experimental hosts showed a morphological identity with similar human neoplasms, but because of the extreme variation in biological behavior in the two species it was concluded that the rabbit tumors are not analogous with the well defined clinical and pathological entity of Wilms' tumor in man.

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DESCRIPTION OF FIGURES 10 TO 15

Fig. 10.—Section of another area from the same tumor showing larger and smaller tubular epithelial structures with a minimum of connective tissue elements. Mag. × 32.

Fig. 11.—Higher power view of previous section. Mag. × 85.

Fig. 12.—In several small areas the tumor was made up of masses of small round cells in solid medullary arrangement. Mag. × 85.

Fig. 13.—First generation anterior chamber transplant of tumor from rabbit X6559. Section was obtained from a biopsy performed 269 days after transfer. All the transplants were made up of well differentiated epithelial tubules, and the characteristic spindle cells and small round cells of the primary tumor were never found. Mag. × 104.

Fig. 14.—Fourth generation anterior chamber transplant of X6559 tumor 671 days after transfer. Cystic degeneration of the tubules was a common finding in old transplants of this type. Mag. × 85.

Fig. 15.—Second generation of testicular transplant of X6559 tumor 168 days after transfer. The morphology of anterior chamber and testicular transplants was identical in all cases. Mag. × 85.

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Mammary Cancer in Fostered and Unfostered C3H Breeding Females and Their Hybrids*

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(Received for publication March 5, 1943)

The C3H strain of mice was started by Strong (16–18) in 1920. The parental animals were a female of the dilute brown stock and a male of the Bagg albino strain. Mice of this strain have been selected for the black agouti coat color and a high incidence of spontaneous mammary carcinoma.

Strong has not published complete data on the incidence of mammary cancer in the animals of his colony but it is known to be high.

In 1933, Andervont assumed supervision of the colony of C3H mice maintained at the Public Health Service. Animals to start the line had been obtained from Strong in 1930. In his first report, Andervont (3) stated that the incidence of mammary tumors in breeding females was 76 per cent (642 mice). Several sublines were being continued and Andervont finally selected line 876 as it had the earliest average tumor age. All his animals are descended from this group.

The data presented by Andervont over a period of years (1–5) are summarized in his most recent publication (2). The incidence of mammary tumors observed in virgin females of the 12th to 21st inbred generations was 97.4 per cent, the average age 10.3 months. The total number of breeding females observed was 1,451 and of these 91.4 per cent died with mammary cancer at an average age of 8.6 months. Breeding females of the 12th to 21st generations had an incidence of 97.5 per cent. The lower incidence observed in the total number was due, according to Andervont, to the various changes in the location of the colony during the period they were under observation; many mice of cancer age died without cancer while the mice were being moved from one laboratory to another.

As Andervont found that tumors arising in mice of his subline of the C3H stock would not grow progressively when transplanted into mice of our C3H strain, he considers his a separate subline of C3H mice and warns that they should not be used as controls for data obtained on mice from any other colony of C3H mice.

Strong's strain of C3H mice is descended from female 59018 by her daughter No. 63673 (16–18); the present author's line of C3H mice is descended from female 59019 through her daughter No. 66072.[1]

In 1935, the tumor incidence for 200 breeding females was 78.0 per cent and the average tumor age 10.7 months (6, 13). A selected subline of mice, representatives of the 23rd to 46th inbred generations, had an incidence of 92.1 per cent and an average tumor age of 10.0 months (10). Virgin females have not been observed.

Mice of the author's line of C3H mice were found by Suntzeff and his associates (19) and Burns and Schenken (14) to have, in breeding females, an incidence of 69.6 per cent (46 animals) at an average age of 12.2 months. Visscher and his associates (21) recorded that of 48 virgin females surviving to the age at which the earliest tumor appeared 32, or 66.7 per cent, had developed tumors by the 16th month and 15 were living at that age. Or, of the 32 mice that died before the 16th month 31, or 97 per cent, had developed mammary tumors.

A reduction in the incidence of mammary cancer has been obtained by fostering the young born to C3H mothers of the susceptible strains soon after they are born on females that had not the active milk influence for mammary cancer (4, 5, 8). A reduction in the incidence has also been secured by giving the animals a calorically restricted diet (21), which apparently changes the amount of hormones secreted in the animals thus treated.

While it is evident that the females of all sublines of the C3H stock have a high incidence of mammary cancer, variations in the incidences and average cancer ages have been reported. These findings denote that when mice of the C3H strain are used the experiment

* This investigation was completed at the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, and assisted by grants from The Jane Coffin Childs Memorial Fund for Medical Research and The National Cancer Institute.

[1] In previous publications (6, 10, 13) we have referred to mice of our C3H strain as the "Z" stock. The main purpose in doing so was to simplify the designation of hybrid animals, thus, C3H × C57 black mice would be ZbF_1 instead of C3H × C57 black F_1.

must be controlled by mice of the same subline, and that the controls and experimental groups must be observed during the same period for accurate comparisons.

RESULTS

The data on the incidence of spontaneous mammary cancer observed in breeding females of the fostered and unfostered (control) groups of mice of the C3H stock and their hybrids are recorded in Table I. In this communication, as in previous publications, the C3H stock is also referred to as the "Z" stock. All the animals received the same care and diet (Purina fox chow).

The unfostered, or control, mice were members of the line with a high incidence of mammary cancer and they were allowed to nurse their C3H mothers.

The fostered mice of the C3H stock were descended from 3 litter mates of the 44th generation that were nursed from the day of birth on a female of the low cancer C57 black stock (8). The progeny and descendants of these mice nursed their mothers; only three were nursed by females of the C57 black, or B, stock. The mice of the fostered group on which observations have been completed would be representatives of the 45th to 50th generations and might be compared with the animals of the unfostered group of the 45th to 51st generations.

The incidence to date of tumors for mice of the fostered C3H group has been 1.2 per cent (2 of 168). Whereas the average cancer age for the unfostered mice of comparable generations was 8.9 months, the noncancerous mice of the fostered group survived to an average age of 16.9 months (Table I). The 2

TABLE I: INCIDENCE OF MAMMARY CANCER AND THE AVERAGE CANCER AGE IN BREEDING FEMALES OF THE Z, OR C3H, FOSTERED AND UNFOSTERED FEMALES AND THEIR HYBRIDS

Stock	Generation	Nursed by	Number	With cancer	Without cancer	Cancer, per cent	Average age: With cancer	Average age: Without cancer	Cancer incidence,* per cent
Z	F_{23}–F_{36}	High cancer Z	227	202	25	89.0	11.2	10.1	96.7
Z	F_{37}–F_{44}	High cancer Z	164	148	16	90.2	10.1	9.1	97.4
Z	F_{45}–F_{51}	High cancer Z	214	208	6	97.2	8.9	9.2	98.1
Z	Total	High cancer Z	605	558	47	92.3			97.4
Zb	F_{44}–F_{50}	C57 (B) ♀†	168	2	166	1.2	17.5	16.9	
$ZZbF_1$	Z♀ × Zb♂	High cancer Z	138	129	9	93.5	9.6	9.4	97.0
$ZZbF_2$	F_1 *inter se*	$ZZbF_1$	136	128	8	94.1	9.4	8.3	98.5
$ZbZF_1$	Zb♀ × Z♂	Low cancer Zb	146	14	132	9.6	10.2	16.6	
$ZbZF_2$	F_1 *inter se*	$ZbZF_1$	275	25	250	9.1	9.1	14.9	

* Omitting mice that died without cancer before reaching the average cancer age.
† Descended from 3 litter mates fostered by C57 black female, only mice fostered.

Because of variations in the average tumor ages the animals have been grouped by generations: mice of the 23rd to 36th inbred generations with average tumor ages, by generations, ranging from 10.4 to 14.9 months; representatives of the 37th to 44th generations with tumor ages consistently under 11 months; and mice of the 45th to 51st generations where the average tumor age for any generation did not exceed 10 months. The average ages, by generations, are represented in Fig. 1.

The incidence of mammary tumors increased from 89 per cent for mice of the 23rd to 36th generations to 97.2 per cent for those of the 45th to 51st generations, with a decrease in the average tumor age from 11.2 to 8.9 months. The incidence for the entire population was 92.3 per cent.

Females of the susceptible unfostered line that died *without cancer* had progeny and descendants with an incidence of 91.8 per cent. The oldest noncancerous female in the group died at the age of 514 days. Two daughters and 4 granddaughters were continued as breeding females; all died with mammary cancer.

females that developed cancer had none of their progeny continued.

To determine what the incidence of mammary tumors would be in hybrids, reciprocal matings were made between animals of the fostered and unfostered groups of C3H mice. To simplify the designation of the hybrids, mice of the susceptible or unfostered group were called Z animals; those of the fostered or low cancer group were called Zb (Z fostered on B females). When control, high cancer females of the Z stock were mated to males of the fostered Zb line, the resulting hybrids were called $ZZbF_1$; the mice obtained by reciprocal matings (fostered Zb females by unfostered Z males) were termed $ZbZF_1$ hybrids. The mice of the second filial generations were derived by mating the mice of the respective first generations *inter se*. All the hybrids nursed their maternal parents.

The hybrids descended from unfostered females of the high cancer line ($ZZbF_1$ and $ZZbF_2$) had incidences of 93.5 and 94.1 per cent, average ages 9.6 and 9.4 months. The mice of the $ZbZF_1$ and $ZbZF_2$ generations showed incidences of 9.6 and 9.1 per cent

respectively (Table I). The average cancer ages were similar to those observed for the mice of the control stock and their hybrid animals (10.2 and 9.1 months), but the noncancerous mice survived much longer than did the cancerous animals of these and other classes with a high incidence.

Mice of the two sublines and their hybrids are compared in Fig. 2. This chart combines the unfostered mice and the hybrids having maternal parents from the unfostered line and the fostered mice and their maternal hybrids. Two curves represent the percentage of the total living to the beginning of each age period or longer. The cancer curves are based on the percentage of animals living to the beginning of each age period or longer, to die with mammary cancer. From this chart it is evident that the mice of the fostered group and their hybrids survived much longer than did the animals of the control series and their hybrids, but that only a small number of the fostered group developed mammary cancer.

Fourteen mice of the $ZbZF_1$ generation were observed to develop mammary cancer while 25 cancerous mice were obtained in breeding females of the $ZbZF_2$ generation. All these cancerous animals were the progeny of, or descended from, 6 fostered females of the Z, or C3H, strain. Only one of these mothers developed mammary carcinoma (416 days); the other 5 died without cancer at an average age of 563 days. No mammary cancer had appeared in the ancestry of any of these females since fostering.

The pedigree for the mice descended from one of these fostered females, No. 71164, is given in Fig. 3. She was mated to a male of the control stock to obtain $ZbZF_1$ hybrids. Two hybrid females born in the second litter to No. 71164 were mated, as were 5 of their progeny of the next generation ($ZbZF_2$). All these mice died without having developed cancer. Five females born in the third and fourth litters to female 71164 were observed as were 12 of their offspring of the following generations. All died with mammary cancer. Litter mates of female 71164, kept in the same breeding pen and mated to the same unfostered male, remained free from cancer as did all their progeny or descendants.

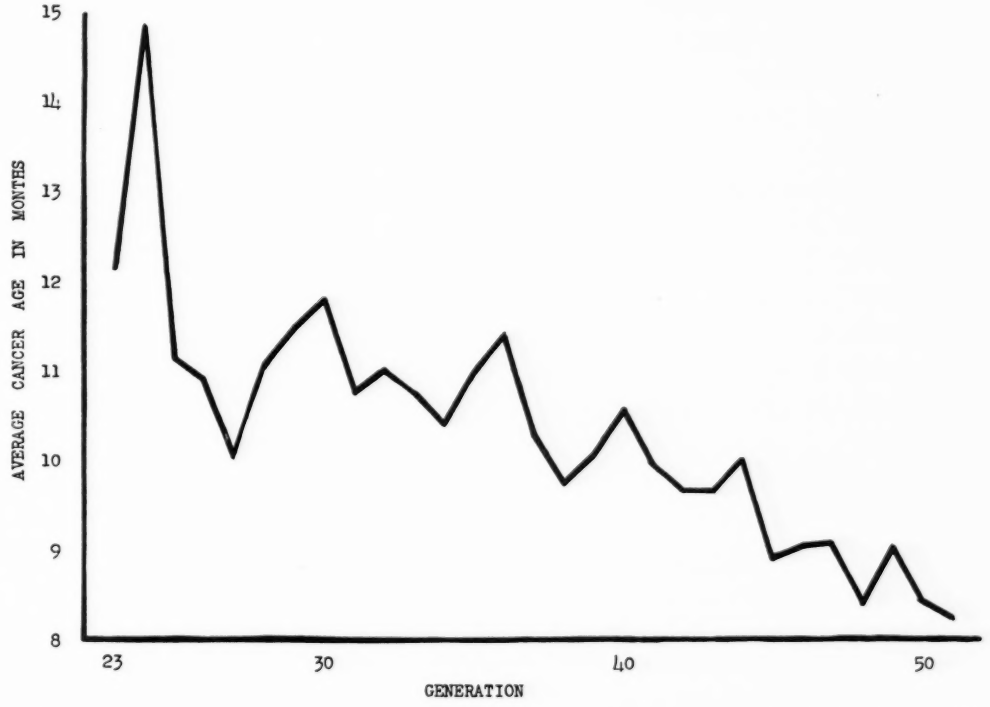

FIG. 1.—Average mammary cancer age in months for successive inbred generations of the Z, or C3H, stock.

The cancerous progeny of the other fostered C3H females are tabulated by litters in Table II. The majority of these females had progeny continued from successive litters (denoted in parentheses). Whereas most of the animals born in the early litters (first and/or second) were free of mammary cancer, those born in succeeding litters developed it and they in turn had cancerous offspring. The cancerous, fostered female, No. 71171, had noncancerous progeny in her second litter but cancerous progeny born in her third litter. The offspring of these mice were also cancerous. First generation hybrids born to female 71484 had no mammary cancer but one of her F_1 daughters had 5 F_2 progeny that developed cancer.

The 39 cancerous mice of the $ZbZF_1$ and $ZbZF_2$ generations were members of litters that had an in-

TABLE II: APPEARANCE OF MAMMARY CANCER IN HYBRID PROGENY OF FOSTERED C3H FEMALES IN SUCCESSIVE LITTERS (LITTERS GIVEN IN PARENTHESES). LITTER MATES OF THESE FOSTERED C3H FEMALES HAD 43 DESCENDANTS CONTINUED; ALL DIED WITHOUT CANCER

Fostered C3H	Hybrids–ZbZF$_1$		Hybrids–ZbZF$_2$	
	With cancer	Without cancer	With cancer	Without cancer
71153 — 631—(2)	0	5	0	9
—(4)	3	1	1	1
71156 — 321—(1)	0	1	0	2
—(2)	1	1	3	0
71157 — 631—(3)	3	0		
71171 + 416—(2)	0	2	0	3
—(3)	2	0	4	0
71484 + 687—(4)	0	4	5	0
71164 — 544—(2)	0	2	0	5
—(3)	3	0	7	0
—(4)	2	0	5	0

milk influence or inciter; (b) the mammary tumor inherited susceptibility inciter; and (c) the hormonal influence that has been termed the mammary tumor estrogenic inciter (7, 12).

Mice of the inbred C3H (or Z) strain have a high incidence of spontaneous mammary carcinoma when the young born to females of the stock are permitted to nurse their mothers with an active milk influence. The noncancerous mice have progeny that also show a high incidence, indicating that in an inbred strain of susceptible mice there is no apparent difference between cancerous and noncancerous animals.

In confirmation of the observations of Andervont (1–5) and previous statements by the present author (6, 10), there may be a lowering of the average age at which the females gave rise to mammary cancer with continued inbreeding. Because the same decrease

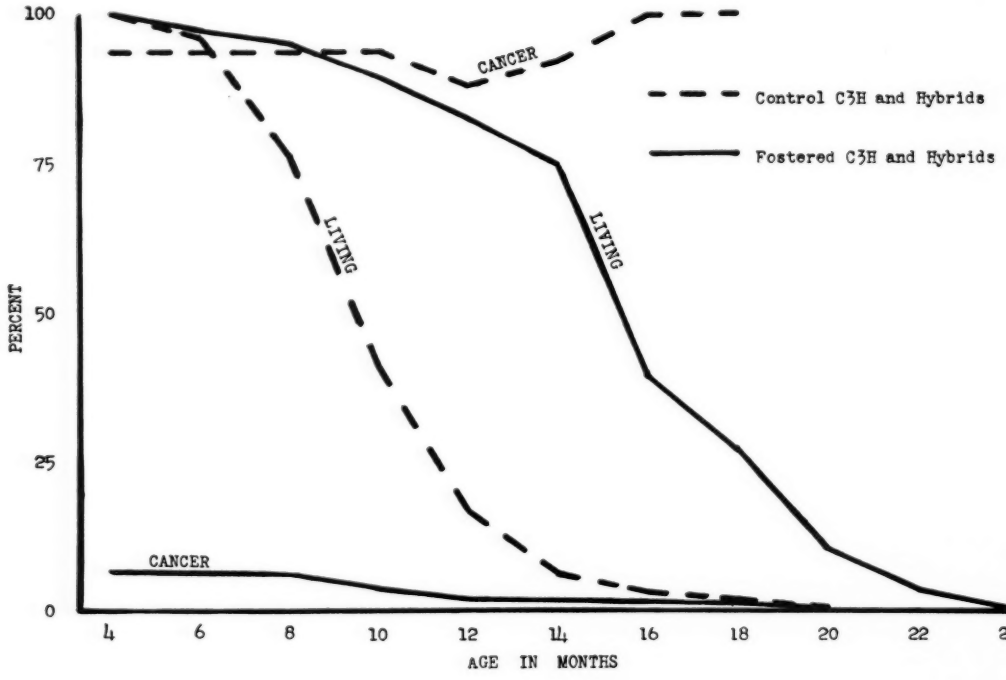

FIG. 2.—Percentage of fostered and unfostered (control) mice of the Z, or C3H, stock and their maternal hybrids living to each period or longer, and the percentage living to each age period or longer, to die with cancer.

cidence of 92.9 per cent. Mice of these litters that did not develop cancer had cancerous young and are included. The parents of these cancerous animals had 33 progeny and descendants born previously to their cancerous offspring and in no case did any have mammary cancer.

DISCUSSION

For the past few years it has been apparent that there are several inciters involved in the development of spontaneous mammary tumors in mice. Three that have been recognized are: (a) the mammary tumor

was noticed also in virgin females of the C3H stock, Andervont (2) stated that selection was probably the main contributing influence.

It is necessary to assume that mice of an inbred strain should be homozygous for any inherited character. According to genetic principles, it has always been maintained that selection within a pure line is without effect. However, living material is subject to mutational changes at any time. In tabulating the average tumor ages by generations it was noticed that when there was a change in the average tumor age it was a sudden one, and that it remained relatively constant for a number of generations.

In addition to the inherited susceptibility for spontaneous mammary cancer, there are other contributing causes that may influence the average cancer age. These are the sensitivity of the mammary tissue to a given amount of estrogenic hormones and/or changes in the level of estrogenic stimulation. Further studies are needed to determine how these influences may be dependent on intrinsic factors.

Another possibility for the changes in average tumor age may be the relationship of the diet to estrogenic stimulation. Although the same commercial food was used throughout the experiments, it is known that the diet was not made with the same formula during the entire period. The modification of the food, with substitution or increases in the proportion of the various constituents, might have altered the amount

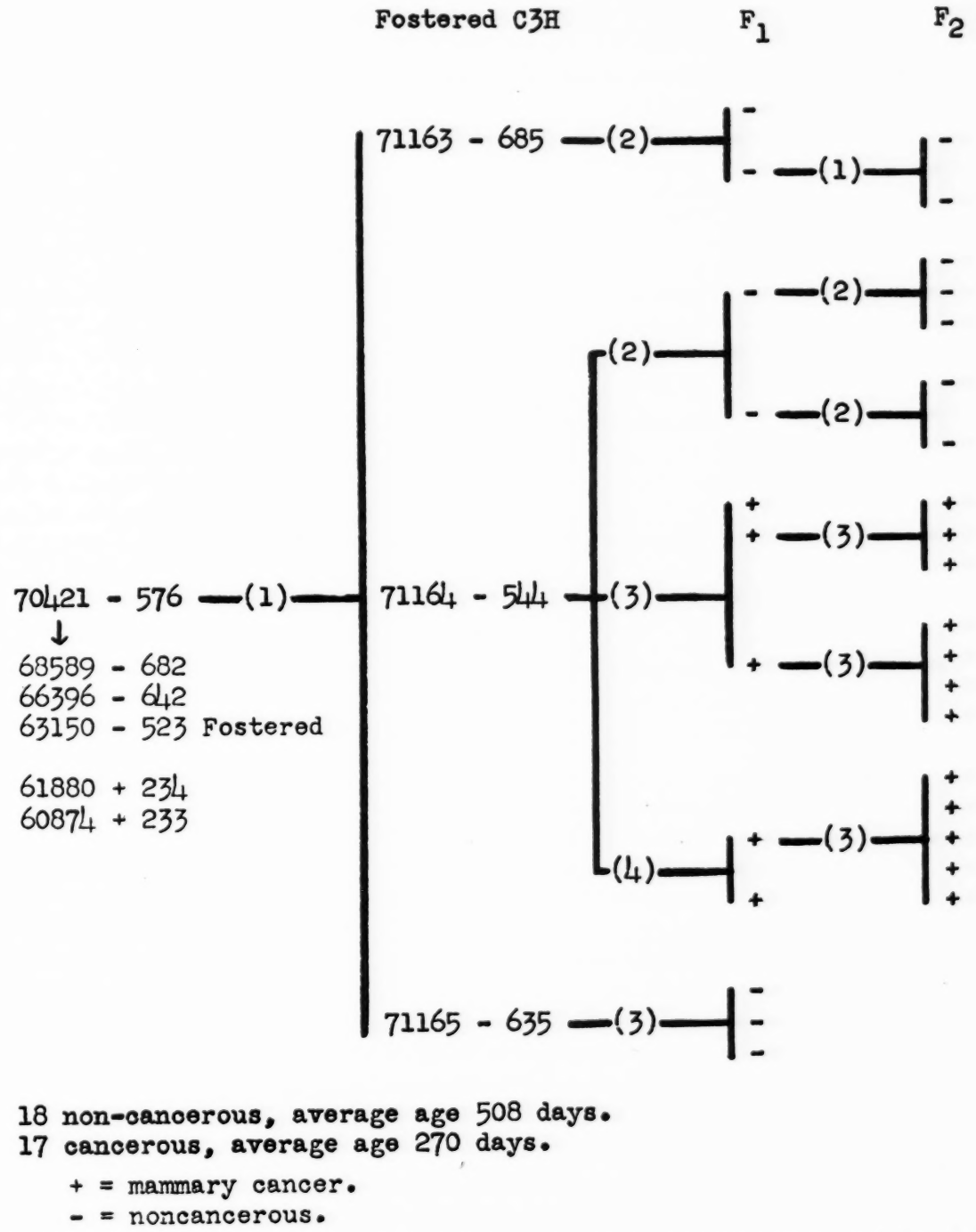

FIG. 3.—Pedigree relationship of the cancerous and noncancerous descendants of fostered C3H female 70421. Numbers in parentheses denote the litters in which the mice were born.

of estrogenic hormones secreted by the animals. If more hormones resulted the average cancer age would probably be lowered. An attempt will be made to determine if the changes in the food corresponded to alterations in the cancer age.

A lowering in the average age at which animals develop mammary cancer may be accompanied by an increase in its recorded incidence, an increase that may be more apparent than real.

In determining the incidence of mammary tumors in a strain of mice, it is usually customary to include in the tabulations all the animals living to the age at which the first (earliest) tumor is noticed. The incidence is the percentage of mice living to this age that develop mammary tumors. Mice of strains, or sublines of the same strain, with a late tumor age would have to survive longer for tumors to develop, on the average, than would those of strains with an early average tumor age. If the average tumor age were late, more animals would have the opportunity to survive beyond the time at which the earliest tumor was recorded but to die without cancer before attaining the average cancer age.

As Table I indicates, the C3H mice born in the 23rd to 36th inbred generations had a lower incidence of mammary tumors (89.0 per cent) and a later tumor age (11.2 months) than did the mice belonging to the 45th to 51st generations (97.2 per cent and 8.9 months). The difference in incidence was 8.2 per cent ($3.4 \times$ S.E.), based on the percentage of mice living to the fourth month or the age at which the youngest mouse of each group had mammary cancer. In the earlier generations the mice had to live longer to attain the average cancer age and so more of them had the opportunity to die without cancer. If noncancerous mice dying before the average cancer age for each group are not included, the respective incidences would be 96.7 and 98.1 per cent (the difference equals $0.9 \times$ S.E.). In one comparison the difference is significant; in the other it is not.

The mice of the C3H stock born to females of the high cancer line but fostered soon after birth on females of the C57 black strain showed, with their descendants, a very low incidence of mammary cancer. The noncancerous mice of the fostered group lived 8 months beyond the cancer age of the control or unfostered mice.

The mating of females of the unfostered line (susceptible) of C3H mice to males of the fostered C3H stock gave hybrids of the first and second generations that had approximately the same incidence and average tumor ages as did the mice of the control group. As there was no evidence for the segregation of inherited susceptibility factors in mice of the second generation, the data confirm previous observations (11) that foster nursing does not alter the inherited susceptibility for the development of mammary cancer.

Hybrids derived from reciprocal matings (fostered females by unfostered males) gave incidences of 9.6 and 9.1 per cent for the first and second generations. The mothers of these mice had a lower incidence of mammary tumors. All the cancerous hybrids were descended from 6 of the 30 fostered C3H females that were mated. Five of these 6 parents died without cancer. Whenever it was possible to make comparisons by the use of pedigrees (the progeny test), it was observed that some of the mice born to these females, by litters, were noncancerous whereas all the mice born in other litters developed cancer. It was noticed also that the litters in which the noncancerous mice were born were always cast before the litters having cancerous mice. The cancerous mice had cancerous progeny and the litter in which these progeny were born made little difference. In most instances the litter mates of the fostered C3H females that had cancerous progeny did not develop mammary cancer nor had they cancerous young, although they were kept in the same pen and were presumably subjected to the same influences.

In a previous publication (9) the occurrence of a mammary cancer in a female of the fostered A stock was described. Progeny of this female had been mated and they in turn developed cancer. From them a line of mice was obtained that have the same incidence of mammary cancer as mice of the unfostered A stock. The litter mates, and their progeny, of the original female that became cancerous remained free of cancer. To explain the development of mammary cancer in this line of mice it was assumed that the active milk influence had appeared *de novo*.

According to van Gulik and Korteweg (20) the active milk influence plays a role, in conjunction with intrinsic factors, in determining the architecture of the mammary glands when they develop following stimulation by the estrogenic and associated hormones. This work has been corroborated by others (15).

It has been demonstrated that mice susceptible to the development of mammary cancer but not having the active milk influence will develop mammary cancer if they receive the active agent, either by injection or feeding, at least up to the time they are 6 weeks of age. The period probably extends to the time when the mammary glands develop. Preliminary work (12) done in association with Duran-Reynals indicated that if mice of the same strain or strains with the same genetic constitution were permitted to have several litters of young before being given material with the active milk influence they generally remained free of cancer.

In the present experiment only 1 of the 6 fostered

C3H females that had cancerous progeny was herself cancerous. Neither she nor the others transferred the active milk influence to their progeny born as members of their first and/or second litters. In some cases the active milk influence developed between the first and the second litters; in others between the second and third litters, etc.

From the work of van Gulik and Korteweg (20) and Shimkin, Grady, and Andervont (15) it may be assumed that the architecture of the mammary glands of these mice, as well as the older ones mentioned above (12), was developed in the absence of the active milk influence for the development of spontaneous mammary cancer. When the active milk influence was present, either *de novo* or as the result of injection, it was impossible for it to alter the structure of the pre-noncancerous gland to a precancerous gland. As a result, few became cancerous, although they were able to transfer the active milk influence to their progeny while nursing. Since the progeny received the active milk influence while nursing, their mammary glands evidently developed with the precancerous architecture as they showed a high incidence of mammary cancer.

This study also emphasizes again the fact that the active milk influence must be considered to be a preparatory inciter in the development of mammary cancer in mice. This preparatory influence is best seen in the determination of the architecture of the mammary glands, and it must be present from the time the glands start to develop, months before the time for the mammary tumor to appear. No single influence may be considered the "inciter" for mammary cancer in mice from evidence available at the present time. Mammary tumors usually result only when the associated influences or inciters are present and active (7, 12).

CONCLUSIONS

As previously demonstrated, foster nursing does not alter the inherited susceptibility to the development of spontaneous mammary carcinoma in mice.

The active milk influence may appear at any time in the life of a mouse. If this occurs in animals whose mammary glands have developed in the absence of the active agent, spontaneous mammary cancer usually does not result.

Females that have the active milk influence and do not develop mammary cancer may transfer this influence by nursing to their progeny.

The average age for the development of mammary cancer in mice of an inbred strain may change with continued inbreeding. The incidence of mammary cancer may be influenced by the average cancer age.

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Studies on the Effect of Hypothermia

I. Acute Physical and Physiological Changes Induced by the Prolonged Hypothermic State in the Rabbit*

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This paper deals with the physiological changes occurring in the normal and the tumor-bearing rabbit (the Brown-Pearce rabbit epithelioma) during lowered body temperature states (hypothermia) at levels between 10° C. and 28° C. While experiments in rabbits cannot be said to duplicate the results that occur in the human body during exposure to cold (5, 7, 8, 12, 13) or to the therapeutic use of prolonged hypothermia near 27° C. (3, 10), yet the changes produced in the rabbit are very similar to the effects of therapeutic hypothermia noted in our limited clinical experience. In general, too, the basic results are similar to the observations at higher levels on the cat (2, 9), yet this is the first time that an effort has been made to maintain an animal at a predetermined low temperature for any prolonged and therapeutic period. The technic is simple and the range of safe temperatures below normal is very much wider than is the case in prolonged hyperthermia, where the safe upper limit is only 5° C. above the normal. The resources of the animal are spared after the initial fall of body temperature, while in the febrile state they are more rapidly and continuously used up as the temperature rises (6).

The body of the warm blooded animal is maintained at a rather constant temperature level (13, 14) that predicates a certain speed for the functioning of body processes. Some animals are so designed that a state approaching suspended animation (hibernation) can be brought about spontaneously with the onset of cold weather or after prolonged exposure to cold, during which the body temperature falls to low levels and rises spontaneously to normal as environmental temperatures rise again (1, 4, 11). The rabbit does not respond to this seasonal change, yet a pseudohibernating or equilibrium state can be produced artificially and maintained for some time (at least 48 hours) with recovery. The recognition of exhausted and preagonal states is difficult. Little attention is given to this in the literature. Since there seems to be a close relationship between our findings in rabbits and clinical experience with therapeutic hypothermia, we are reporting certain of our observations in some detail to emphasize the dangers in the hypothermic state as well as to indicate some of the resources that the body maintains at low temperatures.

METHOD

Seventy-five young adult, healthy, white male rabbits of the New Zealand strain weighing from 2 to 3 kg. each, were subjected to varying degrees of hypothermia. Fifteen were normal and were carefully studied for their reactions to the various procedures. Sixty had received intratesticular inoculations of the Brown-Pearce rabbit epithelioma from 1 to 2 weeks previous to the hypothermic experiments, and were subjected to hypothermia as a therapeutic procedure. The details of the results are reported in Papers II, III, and IV. The response to the hypothermic state of those animals that had the tumor was the same as that of the normal animals. They were included only to indicate that hypothermia can be utilized on a fairly large scale. The animals were not fed during the treatment, but before and after the hypothermia they were kept in wire cages with food and water always available. No type of anesthetic was employed during these experiments because of the rapid soporific effect of the hypothermia.

A multiunit needle and rectal thermocouple apparatus with a range of 0–60° C. was employed for the temperature measurements. A thermocouple was introduced 5 to 7 cm. into the rectum, so that it was possible to take continuous temperature readings throughout the experiment, uninfluenced by the environment (14).

Various modifications of a rather simple procedure for lowering the body temperature were used. The

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hair of the rabbit was thoroughly wet under a faucet and the animal was placed in a small cylindrical wire cage that was then used to immerse the animal in a vertical sitting position, chest high in a tank of ice and water at 0° C. After the rectal temperature of the animal had been lowered to the desired degree, the cage containing the rabbit was removed from the ice water and placed on a table at room temperature (24–28° C.), or in a combined radiant heat-refrigerating cabinet where air temperatures of 0° C. up to 60° C. could be maintained. For maintaining the temperature the tank water was drained off, the tank drain left open, and more ice packed about and over the cages (Table I). The melting ice usually produced enough water to keep the fur of the animal wet, for if the hair dries the body temperature will rise even at an air temperature of 10° C. Rabbits could be kept in this cooled environmental temperature or in the refrigerating unit for 24 to 48 hours or longer, and the body temperatures controlled fairly well (±3° C.) simply by adding more ice if the temperature rose or by removing the wire cage to a table at room temperature if the rectal temperature fell too far. Four to 6 rabbits could be treated at the same time in this manner. The "nursing" problem rapidly became more difficult if the number was increased.

Table I: Body Temperature at Sacrifice after Continuous Immersion of Lower Half of the Trunk in Ice and Water (See Text)

Rabbit Time of reduction	No. 1 6 hours	No. 2 5½ hours	No. 3 5 hours
Rectal	10.4° C.	16.4° C.	15.8° C.
Liver	12.5° C.	18.5° C.	21.0° C.
Deep peritoneal	12.0° C.	18.0° C.	20.0° C.
Lower lobe of left lung	12.5° C.	19.0° C.	—
Mouth	11.0° C.	16.4° C.	—
Testicle tumor	Below 10° C.	Same	Same

EXPERIMENTAL OBSERVATIONS

Induction.—There was considerable individual difference in the reaction of various rabbits to reduction of body temperature, although the sequence of changes was fairly constant. Immediately after the animal had been immersed chest high in cold water at 0° C., the ears became a dull bluish-white and the temperature of the ear skin and cartilage dropped as much as 5–10° C. in different animals (Table II). The thoracic hair became erect, although wet. The animal usually remained very quiet and appeared entirely unaffected by its environment for from 15 to 30 minutes, during which there was a drop in the rectal temperature of about 2° C. Shivering then commenced and a certain amount of restlessness was noted. When the animal's temperature was rapidly lowered, shivering was observed in some cases even when the rectal temperature was in the vicinity of 10° C. In others, the animal soon became unconscious and remained flaccid and limp without shivering (at temperatures between 20° and 10° C.). The deep reflexes disappeared below 20° C. (9), cyanosis developed in the sclerae, and the white skin became bluish.

Table II: Temperature Changes during Hypothermia

Location	11:30 a.m. ° C.	11:45 a.m. ° C.	12:00 noon ° C.	12:10 p.m. ° C.	1:25 p.m. ° C.	2:00 p.m. ° C.	3:00 p.m. ° C.	4:00 p.m. ° C.	5:00 p.m. ° C.	5:50 p.m. ° C.	7:30 p.m. ° C.	12:15 a.m. ° C.	2:30 a.m. ° C.	9:10 a.m. ° C.
Subcutaneous—lumbar	37.8	37.9	34.0	32.1		34.3	33.5	33.3	33.0	32.8	32.3	27.5	26.4	26.8
Deep muscle—lumbar	38.1	37.8	34.5	33.7	29.5	33.5	32.5	32.8	32.6	31.9	27.5	26.3	26.3	26.3
Intraperitoneal—lateral	31.5	29.6	31.0	33.3	32.6	33.5	33.5	32.9	33.5	33.1	32.7	28.0	27.5	27.1
Left lung—base posterior	38.9	38.8	38.4	38.3	35.2	35.0	33.6	33.8	33.5	33.1	32.5	28.2	27.6	27.1
Thigh muscle	39.1	39.0	38.9	38.8	35.3	35.3	33.5	33.8	33.5	33.0	31.7	27.0	26.9	26.4
Intraperitoneal—deep	39.0	39.0	38.8	38.7	35.4	34.9	33.5	33.2	32.7	32.8	32.0	27.8	27.2	26.5
Left testicle	35.7	35.0	32.4	O.S.*	O.S.*	O.S.*	O.S.*	O.S.*	O.S.*		O.S.*	O.S.*	O.S.*	
Left ear cartilage	31.9	30.8	25.0	O.S.*	O.S.*	O.S.*	O.S.*		O.S.*		O.S.*	O.S.*	O.S.*	
Rectal (7 cm. depth)	39.0	39.0	38.5	38.2	33.5	33.8	31.5	31.5	32.0	31.8	30.7		26.5	26.2

No anesthesia. Immersion of lower two-thirds of trunk in ice and water until 12:15 p.m. Animal then kept in a refrigerator in a cage covered with ice (see text).

* Off scale (below 20° C.).

Several animals with temperatures below 20° C. developed repeated generalized convulsions of a clonic type which lasted from ½ to 1 minute. Following their disappearance the animal would again become flaccid. The prognosis for recovery in such cases was usually unfavorable.

Mortality was greater during the induction period and death was often sudden. A too rapid fall in temperature was not well tolerated. Extreme restlessness, sudden respiratory stridor, and extreme cyanosis also were danger signs during this period.

After reduced temperatures, autopsy showed little more than that the vessels were bluish, the blood dark, viscous, and very slow to clot. Little blood drained into the thorax after severance of the great vessels and pulmonary tree. Most of the organs appeared dry and contained little free flowing blood, except for the thyroid, the changes in which are described elsewhere. Apparently the viscosity was so high that the work of the heart was tremendously increased and the blood flow greatly diminished, although the latter was not

measured. It was very difficult to obtain blood samples for chemical analysis either from the ear vein or by cardiac puncture at these temperatures. Feces were passed during all the unconscious period, but no urine was voided during hypothermia.

Passive status resembling acute hibernation or pseudohibernation.—If the animal's temperature was lowered in 3 to 4 hours to the vicinity of 20–23° C., and then permitted to rise to and maintain a level of 23° to 28° C., the animal went into what appeared to be a passive or "acute pseudohibernating" state. Its response to external stimuli was practically absent. For the most part it remained in what appeared to be a deep sleep, snoring loudly with a long, coarse, rasping note. The pulse rate was irregular and slow, and often imperceptible. There seems to be a temperature equilibrium at this level at which the body can maintain itself and from which it can recover. When the environmental temperature was elevated to the rabbit's body temperature (20–23° C.) the animal slowly regained normal temperature. It is important to differentiate this artificial state of hibernation from those states where the animal was rendered unconscious by extremely low body temperatures (15–10° C., at which level the tissues seem congealed) or by exhaustion.

Body temperature.—There was a good deal of variation in the rate of fall and recovery of the temperature of different parts of the body, depending upon the opportunity for heat loss, blood circulation restrictions, and the effect of muscle activity. The temperature in the testicles, tumor, and the subcutaneous tissue over the trunk dropped precipitously when the body was immersed in the cold water, but when the animal was again exposed to room temperature or to a dry low temperature environment, these rapidly rose to the vicinity of the rectal temperature soon after the hair dried. The outer portion of the peritoneal cavity is not well insulated and the temperature there likewise dropped precipitously (Tables I, II, and III). The deeper portions of the peritoneal cavity and the deep organs showed a similar although slower change in temperature. The liver remained the warmest organ in the body, with deep muscles and brain slightly cooler (Table III). The rectal temperature was usually the lowest of the deep temperatures, probably because of the cooling produced by the cold blood returning from the lower extremities through this region.

Recovery.—The major part of this study was made during the longer periods of spontaneous or assisted recovery, starting immediately after the lowest temperature had been reached (after 2 to 5 hours of immersion) or after prolonged maintenance (8, 12, 24, and 48 hours) at a fairly low temperature. The individual variations in recovery were numerous if the animals with rectal temperatures between 10° and 20° C. were placed in room temperature at 26–28° C. or if recovery was accelerated in the heated cabinet or in warm water at 35–45° C. In general a steady rise of 3–5° C. per hour occurred without overshooting when the rabbit was placed at room temperature and the hair allowed to dry. When the rabbit was placed in the refrigerator at air temperature around 10–11° C. and the hair allowed to dry, the body temperature rose at the rate of 1–3° C. per hour to normal, or in some cases even reached febrile levels of 39° C. However, if the same conditions were maintained but the hair was kept wet while the animal was in the refrigerating cabinet the temperature of the animal would rise slowly, only to between 23° and 27° C. Here the temperature would remain,

TABLE III: TEMPERATURE CHANGES IN THE RABBIT DURING HYPOTHERMIA

Location	10:05 a.m. ° C.	11:15 a.m. ° C.	12:15 p.m. ° C.	1:15 p.m. ° C.
Brain—anterior lobe	39.6	31.9	21.8	14.2
Liver	41.0	31.8	23.8	15.8
Left lung—base posterior	40.3	30.6	23.0	15.2
Peritoneal cavity—deep posterior	41.0	31.5	23.2	14.8
Subcutaneous	39.2	32.5	19.4	12.8
Thigh muscle	39.2	30.6	23.2	15.4
Rectum	39.8	30.2	20.0	14.2
Testicle tumor			17.4	

Ice pack and refrigerator started at 10:15 a.m., and continued until 1:15 p.m. Animal sacrificed at 1:20 p.m.

The first temperatures are higher than usual because of the activity of the animal during administration of the anesthetic (nembutal), which was given to permit insertion of the thermocouples.

the animal continuing in the *pseudohibernating state* for any desired period. As soon as external heat was applied, such animals would become conscious and recover rapidly unless they were exhausted or the rise was too rapidly accelerated, in which case death promptly resulted. If the animal with a temperature below 25° C. spontaneously increased its temperature only 3–5° C. or less after 2 hours of exposure to room temperature, death was usually imminent or would occur within the next 4 to 8 hours. Exposure to high temperatures would occasionally resuscitate these animals.

With air or water temperatures of 35–45° C., the temperature *rise* of the rabbit could usually be accelerated considerably, apparently without detriment to the unexhausted animal, although a *fall* of temperature at a rapid rate was usually fatal. Recovery was indicated even before the hair was completely dry by a return of regular respiration, increased and more regular heart rate, disappearance of cyanosis, and a return

of consciousness even though the temperature still was very low (15–20° C.).

After regaining consciousness the animal would lick anything moist and chew aimlessly, but it would not drink either tap water or saline. If the temperature rose steadily the animal would start eating at about 28° C., although at this temperature its movements were uncoordinated and slow. It appeared to react normally at 30–34° C. After recovery most of the animals were ravenously hungry. Several, however, developed diarrhea and failed to eat for various periods of time after recovery.

For the first 1 to 3 days following a prolonged hypothermic state, the rabbit remained somewhat lethargic. If disturbed it appeared to be hypersensitive, jumping quickly away from the disturbance. Five animals had apparently recovered but succumbed 1 to 5 days after prolonged treatment. Two of these had pneumonic consolidations, 1 had a pericarditis, and 2 developed acute nasal discharge and symptoms of snuffles without pneumonic changes or other lesions obvious at autopsy. Established infections or abrasions did not heal well after hypothermia.

When the animal regained its normal temperature following an initial reduction, its capacity to recover from subsequent cooling was reduced. A relatively small reduction in environmental temperature (either air or water) then resulted in a rapid fall in body temperature. After 2 or 3 successive hypothermic states, the ability of the animal to regain its normal temperature was even more retarded or absent. If the rabbit was allowed to recover from the after-effects of hypothermia for a week or more, its reaction to another temperature reduction was apparently no different from that of a stock animal.

The average rabbit can withstand a reduction to 20° C. with spontaneous recovery if the hypothermic state is not induced too rapidly (3 to 4 hours). Below 20° C., however, the ability of the rabbit completely to regain a normal temperature rapidly diminished although some animals could recover from 17–10° C. Below 17° C. the animal usually required additional heating. Without heating, the temperature sometimes rose to 24–28° C. within approximately 12 to 18 hours where it might remain until the animal succumbed 10 to 24 hours later. Of 8 animals that had been lowered to 10° C., 4 recovered by artificial heating, 2 recovered without any additional heat, and the remaining 2 succumbed in spite of artificial heat.

Thus it is obvious that there is no temperature within the range studied that may be described as the lowest from which recovery will occur spontaneously, nor from which recovery may occur with the addition of artificial heat, since many factors determine the response of each animal at these low temperatures.

There was a definite individual variation in the capacity of the animal to delay a hypothermic state as well as in the ability to recover following hypothermia. For example, of 2 animals placed in the same cold environmental temperatures one remained quiet, shivering hardly at all, and from all external signs appeared to make little effort to prevent the reduction of its temperature. The other moved violently about in its cage, and appeared to be resisting the action of the cold. The temperature drop of the first animal, however, was much slower, it withstood its temperature drop better, and regained its normal temperature more rapidly than did the second rabbit. Thus each animal must be treated individually in order to reach a predetermined temperature level and endure it in safety.

Respiration.—The respirations of the rabbit are an excellent guide to its condition. A rabbit in a basal state normally breaths 140 to 200 times per minute. After immersion in an ice bath, if the animal remained quiescent there was no alteration in the rate until the temperature had dropped 1–3° C., at which time there was a decided increase—250 to 500 respirations per minute. As the temperature of the animal dropped below 32° C., there was usually a rapid and corresponding decrease in the respiratory and cardiac rate. At a rectal temperature of 20° C. the respirations were in the vicinity of 60 per minute, and at 10° C. they were sometimes only 5 or 10 per minute, and very irregular. When the animal was in a pseudohibernating state (25–28° C.) the respirations were usually regular and varied from 20 to 40 per minute. During the recovery following short experiments, the respiration rose at the same rate as it decreased during the reduction stage. If it did not rise, the prognosis was usually bad. At temperatures between 10° and 18° C. lapses of a minute or more were sometimes noted between slow deep breaths, or the respiratory movements did not occur for longer periods of time, indicating temporary or final respiratory failure, as the case might be. In most instances, rapid shallow breathing preceded respiratory failure. In some cases both respiration and heart beat appeared to cease for 15 to 20 minutes, yet the animal might recover spontaneously or with heating. Contrary to the observations of Walther (15), edema of the lungs was not found at autopsy, or on histological examination, in any of our animals.

Heart rate.—With the first decrease in temperature (plus excitement), the heart rate increased from 200 to about 270 per minute and then gradually became slower as the temperature fell. With the animal in a pseudohibernating state at 25–28° C., the heart rate was regular, varying between 40 and 70 per minute. At 20° C. the rate was sometimes as low as 20 to

40 per minute. At lower temperatures the heart beat usually became feeble and irregular with frequent missed beats. At times it could not be heard through the chest wall with the stethoscope. An electrocardiogram taken at normal temperature with a heart rate of 270 per minute showed a PR interval of 0.05 seconds, QRS interval of 0.04 seconds, ST interval of 0.4 seconds. Six hours after immersion with the rectal temperature at 20° C., the heart rate varied between 20 and 28 per minute. The electrocardiogram showed a complete block, PR interval not measurable, QRS interval about 0.10 seconds, ST interval as long as 0.40 seconds where measurable, and occasionally ectopic ventricular beats.

Sometimes during extremely low temperatures, the heart beat was fairly loud and strong. In such cases, even though the animal appeared exhausted, the prognosis for recovery was usually good. When the heart sounds were faint and remained so, the animal usually succumbed. Thus the loudness of the heart sounds seemed to be of some prognostic value. When the animal was raising its body temperature the heart rate rose in proportion, although in some cases active effort made the rate unusually rapid.

Delay of death.—When the thorax was opened after the respirations had apparently ceased (temperatures below 20° C.) the heart sometimes continued beating for as long as 2 hours at the rate of 7 to 10 beats per minute. There were frequent dropped beats. The ventricular contraction ceased first. If the thorax was closed to prevent drying and the animal kept cool, the heart might beat spontaneously at long intervals or be stimulated to do so by trauma for 8 to 10 hours longer. In fact it was almost impossible to tell when death did occur at temperatures below 20° C. Not infrequently animals were discarded because "death" had occurred before the planned "treatment" was finished. Such animals were left for disposal overnight in a basket at room temperature. Six to ten hours later, an occasional one of these discarded animals would be found hopping about the laboratory completely recovered. Occasionally an apparently dead animal could be revived in the radiant energy cabinet. In these cases the diminution and disappearance of the vital signs had been gradual as the temperature reached or was maintained below 18° C. (to 10° C.). Disappearance of vital signs following convulsions, sudden respiratory stridor, or excessive physical activity had its customary finality. In such cases, respiratory and cardiac arrest were practically synchronous.

SUMMARY AND CONCLUSIONS

1. Reduction of the body temperature of the rabbit to rectal temperature levels of above 20° C. for therapeutic purposes is readily possible for prolonged periods up to 48 hours with spontaneous recovery.

2. Rectal temperatures between 20° and 10° C. are possible but the ability of the animal to recover spontaneously from these low temperatures is much less and diminishes rapidly at the lower levels.

3. The elevation of body temperature by externally applied heat (radiant energy or warm water immersion) speeds recovery and will often rescue animals that would have otherwise succumbed.

4. Spontaneous recovery from the hypothermic state depletes the animal's resistance to, and ability to recover from, another reduction of body temperature unless several days are allowed to intervene between such states.

5. A pseudohibernation or equilibrium state can be produced in the rabbit by lowering and maintaining the rectal temperature in the range between 23° and 28° C.

6. Spontaneous recovery from this pseudohibernating state is almost always brought about by exposing the animal to room temperature (20–25° C.) with the hair dry.

7. After the initial efforts of the body to compensate for the heat losses, a state of conservation of energy seems to set in and the vital signs diminish proportionately as the body temperature falls until at very low temperatures (10–15° C.) an inanimate state is approached.

8. Death after sudden convulsive seizures, sudden respiratory stridor or failure is sudden and clear cut, but with gradual diminution of the vital signs at low temperatures, objective evidence of death is ill defined and should not be taken as final until efforts to elevate the body temperature artificially have failed.

9. While it is possible to lower the body temperature of a group of animals simultaneously by immersion in ice and water, the body temperatures achieved, the capacity for recovery, and the time required for recovery may vary tremendously from animal to animal.

10. The presence of a 2 week old Brown-Pearce epithelioma did not change the reaction of the rabbit to the hypothermia.

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Studies on the Effect of Hypothermia

II. The Active Role of the Thyroid Gland in Hypothermic States in the Rabbit*

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In rabbits subjected to lowered body temperature (28° to 10° C. rectal temperature) for various periods, an acute profound physical and histological change has been noted consistently in the thyroid gland. Believing that these changes have an important bearing on the ability of the animals, and perhaps of human beings, to withstand periods of reduced body temperature, we are describing them in some detail.

Extensive studies have been performed by numerous investigators (1, 3, 4, 5, 7, 8, 13, 23) on the effect of prolonged exposure to low temperature upon the thyroid gland of various animals, but the results do not always agree. Thyroidectomized animals have been said to suffer more severely in a cold environment than do normal ones (2, 11, 21, 22), yet the contrary is reported also (10). Dietrich and Schweigk (6) recorded an immediate increase of the blood flow through the thyroids of chilled dogs. In a series of extensive experiments, Cramer and Ludford (3) demonstrated that exposure to a cold environment for a short period was a powerful stimulus to the functional activity of the adrenals, but that low environmental temperatures of longer duration were necessary to induce hyperactivity of the thyroid. Ludford and Cramer (15) noted congestion of the thyroid capillaries and increased secretory activity of the follicular cells in epilated rats after 24 hours in an ice chest. Ring (20) observed that in rats exposed to cold for a short period of time the elevation of the basal metabolism was associated with a rise in body temperature. If they lived for 3 weeks or more in an environment of 8–5° C., there was an average elevation in metabolism of 16 to 21 per cent, and the increase in the basal metabolic rate was brought about largely, if not entirely, by the thyroid gland since a smaller increase in basal metabolic rate was obtained in partially thyroidectomized animals.

Seasonal effects on the morphology of the thyroid in birds have been studied by several authors with conflicting results. Haecker (9) described maximal collections of colloid in the follicles in winter with a minimum of colloid in summer in crows and sparrows. Kuchler (14) reported that during the winter the thyroids of robins and sparrows are inactive. On the other hand, Riddle and Fisher (19) believed that the thyroid is most active in winter and Miller (17) agreed, having noted increased thyroid activity in sparrows on lowering the external temperature.

There are many reports indicating the role played by various other glands in heat production; *i.e.,* the adrenal gland, the pancreas, and the brain. Thus many factors may control the reactions of the temperature-regulating mechanism of the body; yet in our studies the thyroid presented the most profound histological changes, and there was no obvious alteration in the adrenals except for the congestion seen in all the organs.

METHOD

Sixty-six normal, young adult, white male, rabbits of the New Zealand strain were used in these experiments over the period September, 1939–March, 1940. They weighed between 2 and 3 kg. each, and had been housed for at least 1 month previous to experimentation in a well lighted, ventilated room at a temperature of 20–26° C. They were fed a diet of alfalfa and oats. Their drinking water was the average city water, with an iodine content of 2 parts per billion. Forty-five of the animals had been inoculated intratesticularly with the Brown-Pearce rabbit epithelioma from 1 to 2 weeks previous to the reduced temperature experiments in order to study the effect of the lowered temperature upon this tumor. Since the general response of the tumor-bearing rabbits to the lowered

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temperature and the observed changes in the thyroid gland were identical in every respect with those of the non-tumor-bearing ones, they have been used in this series of studies for confirmatory data only, the main evidence supporting our observations being obtained from the 15 normal animals subjected to hypothermia. Three normal rabbits were kept without food for 4 days to study their thyroids (Fig. 5 B) and 3 normal rabbits were sacrificed as controls (Fig. 1). The method of lowering the temperature is described in Paper I. The thyroids were removed, fixed in formol-Zenker's fluid, sectioned, and stained by hematoxylin and eosin in the usual manner.

EXPERIMENTAL DATA AND DISCUSSION

Various rates, degrees, and durations of reduction to the lowered temperature level were studied to obtain data on the various possibilities. To facilitate the description and correlation of the changes in the thyroids, the experiments will be discussed in several major groups or series into which they seem to fall both by the nature of the experiment itself and by virtue of the histological changes observed in the thyroid gland.

The normal thyroid gland of the average stock rabbits is a thin, flat, rather flabby, grayish pink structure varying from 1 to 3 mm. in thickness. Each lateral lobe covers an area approximately 0.5 cm. × 1.5 cm. on each side of the trachea. The isthmus is frequently stringy or filamentous in character but may be seen occasionally as a definite bridge between the lateral lobes where it is a thin, flat, translucent structure of ill defined dimensions. The blood vessel supply is clearly defined but not prominent. Histologically there is a good deal of variation in the appearance, size, and distribution of the acini (Fig. 1) yet there is fair uniformity in the cell sizes in a particular gland. The epithelial cells are small and cuboidal. Occasionally in some animals the epithelium is flatter, and more colloid than usual is visible. Occasional vacuoles may be seen at the margins of the colloid.

Since the organs of the hypothermic animals revealed few or no other abnormalities except for a reduced amount of blood, only the details of the thyroid changes will be described.

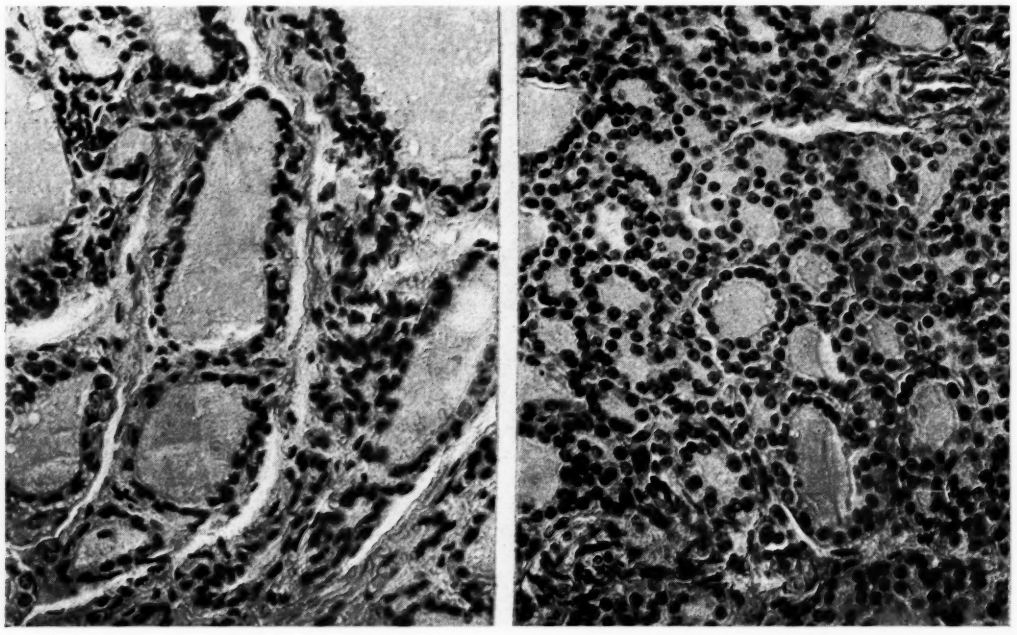
Acute and Fatal Exhaustion State

Six rabbits were immersed chest high in ice and water at a temperature of 0° C. until death occurred (2 to 5 hours). The rectal temperature dropped steadily during this short period, occasionally reaching the lower level of 10–12° C. The thyroid gland was of normal size. All vessels to the thyroid were dilated and engorged. The glandular substance was red but of normal consistency. The cut surfaces showed the usual fine, homogeneous, cellular structure with a slightly waxy surface. Microscopically the follicles varied in size and in their content of colloid from areas where the cellular components constituted the entire follicle with no colloid present (Fig. 2 A) to other areas where the colloid was abundant (Fig. 2 B). The epithelial cells of the follicles were swollen, and varied in shape from the small cuboidal to the almost columnar type. The blood vessels were numerous. There was no evidence of edema.

The histological appearance of the thyroid gland suggests that parts of it were in a state of moderately increased activity. It will be shown later in this paper that it is in this period of the hypothermic state that the metabolic rate is notably increased. Apparently in this acute disturbance of the temperature-regulating mechanism the thyroid was stimulated to a considerable extent, yet because of the short duration of the experiment (2 to 5 hours) it was not able to get well under way (19). The appearance of the gland after this short period of reduced body temperature was similar to that reported by others (3, 23) after almost 2 weeks' exposure of rats to a cold environment.

Occasionally death occurred in a very short period while the temperature was falling rapidly at a rate of 4–8° C. per hour. The animal would become highly agitated and after a particularly violent episode, almost resembling a maniacal state, would suddenly become cyanotic, breathe stertorously, and the respirations and heart would stop. This might occur at any time during the induction period of 1 to 8 hours. Usually in these cases the thyroid would be 1½ to 2 times its normal size, highly congested, and soft. No other gross abnormalities were to be found except for a general congestion of the vessels. The colloid was usually absent and replaced by a small collection of vacuoles. The acini were nearly circular in cross section as if under pressure from inside, and there was more space between them than usual (Fig. 3 A). The cytoplasm of the epithelial cells was swollen, granular, and irregularly stained, and there was often a clear space around the dense and slightly shrunken nucleus (Figs. 3 A and 3 B). This gave the impression that the gland had been acutely exhausted of its colloid after a period of extreme activity and had become edematous. A similar picture was occasionally seen in animals unable to recover spontaneously during the relatively short therapeutic experiments (8 to 10 hours at a low temperature), and in which violent movements aggravated the acute physical exhaustion with resultant death.

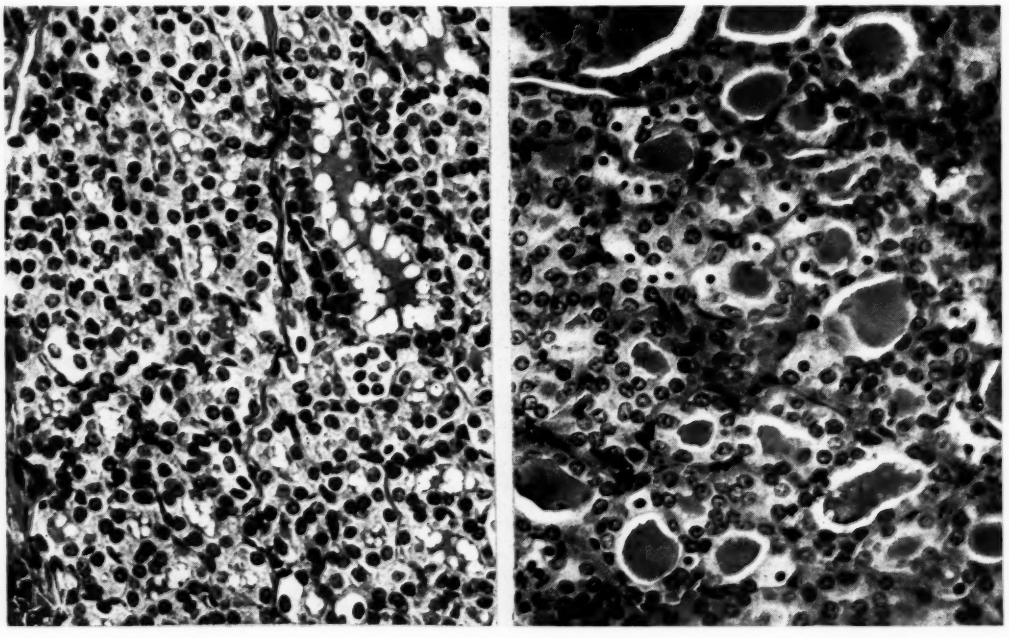
Whether edema in the thyroid cells (Fig. 3 C) was a primary or a secondary factor cannot be determined. Such a condition (rapid exhaustion of the colloid and



A B

All the sections shown in this paper were photographed at the same magnification, × 300.

FIG. 1.—Thyroid glands of 2 normal rabbits showing various sizes and shapes of acini, amounts of colloid, and cell types. The acinar walls in (A) are lined with flattened cuboidal cells containing rather small, densely stained, oval or spindle-shaped nuclei. The epithelial cells in (B) are definitely cuboidal, and the nuclei are round and show some structure. In some fields there are numerous vacuoles and much less colloid. These variations represent the extremes that may be seen in a single gland as well as in glands from different normal stock rabbits during the winter season.



A B

FIG. 2.—Acute changes due to rapid reduction of body temperature resulting in death. The rectal temperature in one rabbit was reduced to 18° C. in 2 hours, when death occurred suddenly and violently. The thyroid was normal in size. Section A shows almost no colloid, the acini being collapsed or containing vacuoles. The acini are small and made up of large, swollen, cuboidal or columnar-cuboidal cells that have a granular appearance. The nuclear detail is clear and there is no definite pyknosis. Another rabbit was reduced to 10° C. in 5 hours when death occurred imperceptibly. The thyroid gland was normal in size. The colloid in (B) is very darkly stained with eosin. The epithelial cells are swollen and columnar in shape. The nuclei are clear and paler than usual.

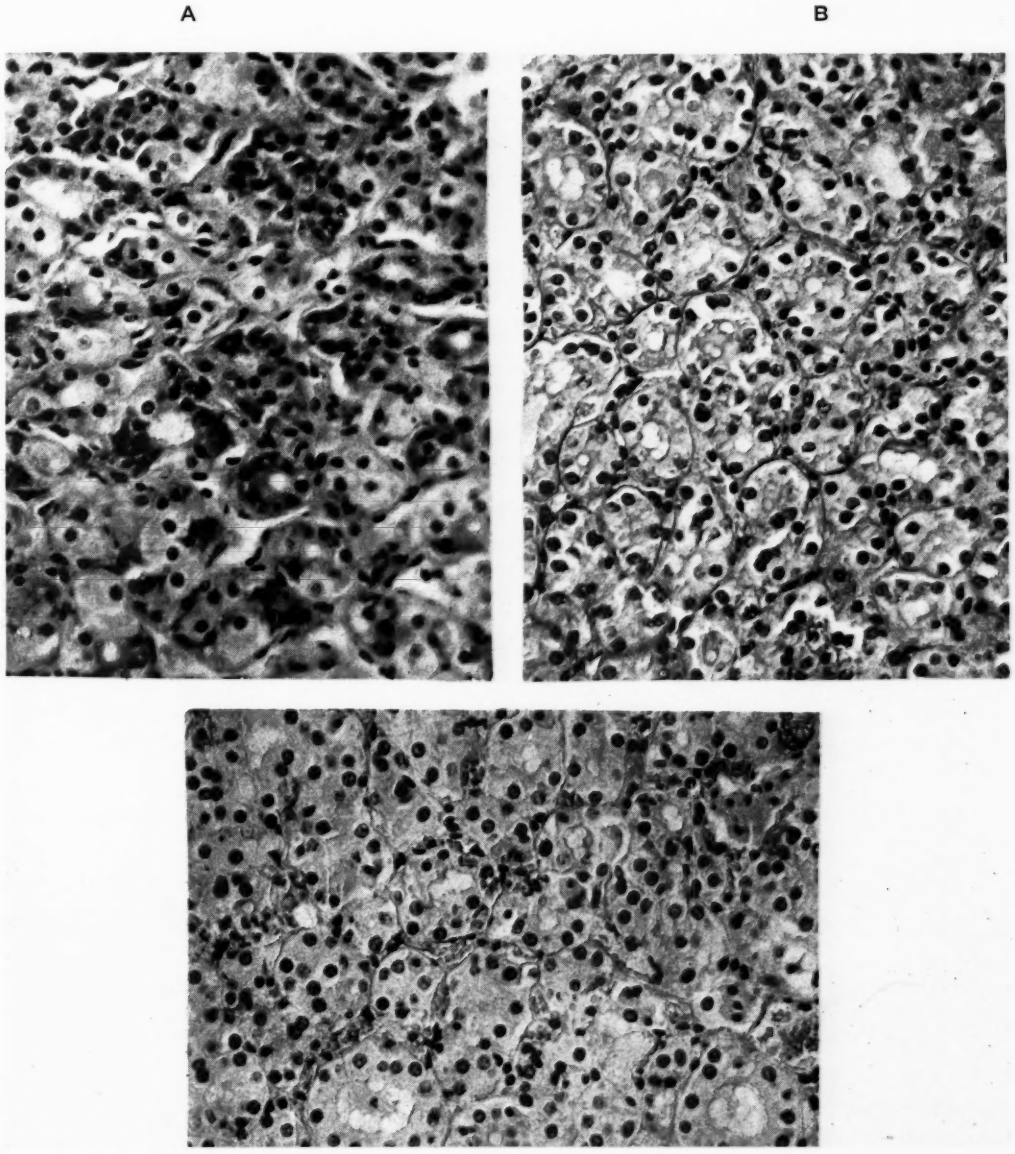


FIG. 3.—Extreme yet characteristic changes in the thyroid resulting from acute exhaustion. Glandular enlargement, cellular enlargement, and beginning cellular disintegration and congestion.

In rabbit 5 the temperature was lowered to 18° C. in 8 hours, when death occurred suddenly. The thyroid gland was 1½ times normal size, congested, and soft. The illustration (A) is characteristic of the large cell size and the small acini almost devoid of colloid or filled with vacuoles. The capillaries, not shown well here, are dilated and engorged with blood. There seems to be considerable free fluid between the acini. The epithelium is swollen and columnar in type, the nuclei being dense and frequently isolated from the cytoplasm by a clear zone. The number of acini seem much greater than normal. The increase in cell size, the edema, and the congestion probably account for the increase in gland size.

Rabbits 6 and 7 were both reduced to a temperature of 18° C. in 6 hours, when death occurred suddenly. The thyroid glands were each enlarged almost to twice normal size, and were soft and engorged with blood. The tremendous cellular enlargement and edema, shown in (B) and (C) are striking. The nuclei are dark and often in various stages of disintegration. This is most prominent in (C) where the cell boundaries are almost lost. Colloid is replaced almost entirely by vacuoles. Small capillaries gorged with red blood cells are seen everywhere. Section B seems to show more actual cell disintegration, while cytoplasmic swelling is more prominent in (C).

swelling of the cells from ingress of fluid) would certainly hinder the normal functioning of the thyroid gland and must have contributed in some respect to the distress and fatal incapacity of the animal to maintain itself.

Evidently some acute hypertrophy (functional) occurred in these glands in this short period since all the enlargement, both in gross and in the histological appearance of the epithelium, was probably not due to edema alone. Similar observations were made on 20 animals that were either sacrificed or that died during the induction period. Some could only partially compensate for the continued loss of heat for 4 to 6 hours. Others succumbed after 10 to 12 hours. In all these brief experiments the thyroid showed a definite abnormality, the degree of which was usually in keeping with either the rapidity of fall of the temperature, the low level reached and maintained for a few hours, or an exhausting violent episode just before death. The temperature at which death occurred varied between 28° and 10° C. The reason some rabbits succumbed when the body temperature was lowered only 8–10° C. while others were able to withstand a drop of 25° C. before death occurred is not known. There seemed to be no difference in the physical condition or in the reaction of the animals to the cold up to the time of death. The soporific effect of the falling body temperature came on rather promptly in most cases.

Spontaneous Recovery Following Reduction of Temperature—Acute Hypertrophy of the Thyroid

Eight animals were reduced to various low levels of hypothermia in order to observe the level from which they could warm themselves spontaneously when kept at room temperature, or from which they had to be aided artificially. Some of these were sacrificed at various stages at the end of the induction and during the periods of recovery, after 6 to 12 hours' low temperature, in order to study the morphology of the thyroid gland in these stages.

The rectal temperature of rabbit 28 at the beginning of the experiment was 38.0° C. It was placed in an ice bath for 6 hours, when the rectal temperature reached 23.0° C. The water was then removed and the cage partially surrounded by crushed ice. When the body temperature reached 17.0° C., 3 hours later, the animal was removed from the ice and placed on a table at room temperature (24.0° C.) It attempted to stand but its movements were slow and uncoordinated, partly because of the shivering that started immediately. This slow muscular activity continued for 3 hours, at the end of which the rectal temperature had reached 35.0° C. The rabbit appeared then to be normal except for a somewhat dull, listless appearance. The hair was dry. The animal was sacrificed at this point after a total of 6 hours at a reduced temperature, and the thyroid gland was studied.

It was approximately 3 times the normal size and its vessels were considerably engorged and dilated. It was bright red in color, greatly congested, and had a hyperplastic, fleshy appearance. Cut section revealed a red, roughened, nonhomogeneous, rather soft surface from which a great deal of blood could be scraped. Histological study (Fig. 4 A) showed that most of the follicles were devoid of colloid with the exception of a few scattered here and there that might contain a little, or even a large amount. Vacuoles were numerous. The cells were large cuboidal to low columnar in shape, their nuclei sharply defined, and the cytoplasmic details were readily seen. The blood vessels were dilated, engorged, and numerous throughout the entire section. There was no evidence of edema. Most of the enlargement of the gland must have been due to actual cellular hypertrophy (3 to 4 times) and blood vessel congestion.

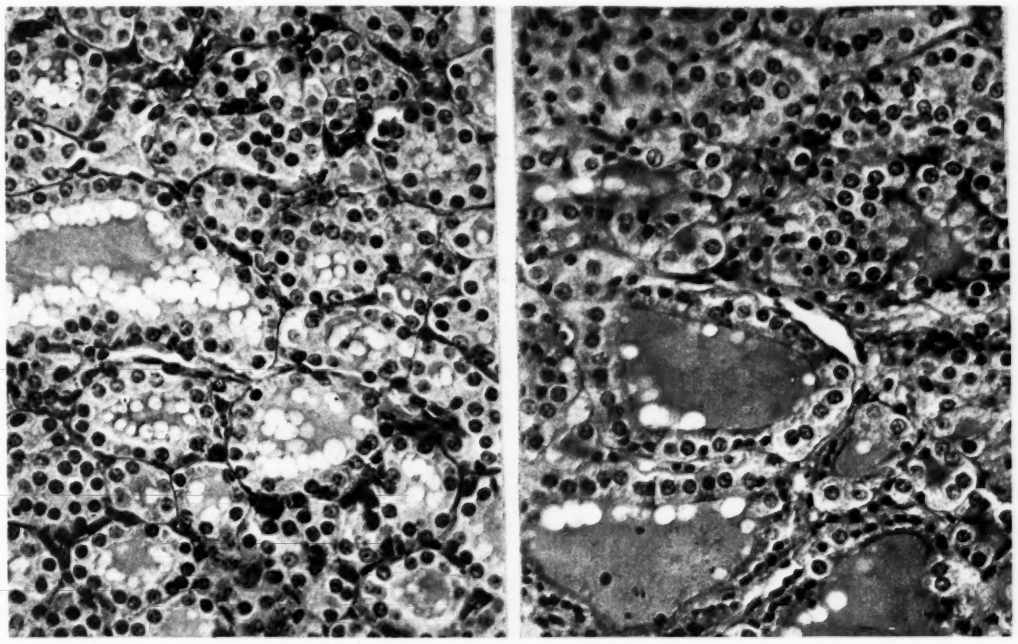
There was evidently a definite thyroid hyperactivity in this stage of the animal's attempt to maintain body temperature. In 5 rabbits the temperature had been lowered to 17.0° C. with spontaneous recovery, and in 3 to 11.0° C., with artificial heating to aid in restoring the temperature to normal. Similar changes or evidences of hyperactivity of this degree were noted in the thyroid glands of all 8 animals. There were no signs of hyperplasia or mitotic activity, probably because of the short duration of the experiments.

As the rabbit starts to raise its temperature from the levels above 20.0° C., the basal metabolic rate has been found to be definitely increased (Table I). This is probably the period at which the thyroid gland is undergoing its greatest stimulus and activity. It is apparently able (8 rabbits) to change the size of its epithelial cells in this short time of 6 to 12 hours or less, and to compensate for the tremendous acute drain that has been thrust upon it. It is interesting to note that this morphological change can occur so quickly. The change is of much greater magnitude than, although similar to, the slower alteration brought about by exposure to low air temperature for weeks (3, 23).

Prolonged Hypothermia or Pseudohibernation—Acute Hyperactivity of the Thyroid

If the temperature of the rabbit was maintained in the vicinity of 28–25° C. or lower, the animal usually remained in a quiescent state resembling hibernation. Several animals were sacrificed at early periods during this state while others were kept in the quiescent,

pseudohibernating state for various periods up to 48 hours. They were then permitted to recover spontaneously at room temperature of 20–26° C., or were aided by artificial means to establish their normal temperature (Fig. 4 A). Some were sacrificed at various periods following recovery and their thyroids studied. Still others were subjected to lower body temperature (21–11° C.) at which point they appeared to be in a state of deep coma or even suspended animation. Spontaneous recovery was possible if this lowered temperature was not prolonged beyond an hour or so (resembled Fig. 4 A). A longer period required artificial heating and a still longer period was usually fatal (Fig. 4 B). The following experiment is typical.



A B

FIG. 4.—Hypertrophy due to stimulus of low body temperature. Spontaneous recovery is possible.

(A) Rabbit 28 was sacrificed after 6 hours at temperatures between 17° and 35° C. (including a 6 hour reduction period). The thyroid gland was 3 times normal size, firm, and engorged with blood. The colloid varies in amount, in some cases being almost absent or replaced by vacuoles. The epithelial cells are sharply defined and large cuboidal or low columnar in type. Blood vessels are numerous and congested.

(B) Rabbit 32 was reduced to 13° C. in 6 hours with spontaneous recovery to 31° C. in 17 hours at room temperature. Additional mild heating for 6 hours more restored the rabbit to normal temperature (36.5° C.), at which time it was sacrificed. The thyroid gland was not enlarged. Some epithelial cells appear swollen and are paler and less sharply defined than others, suggesting the beginning of focal edema and possible disintegration. Some acini have cells on one side that are low cuboidal or flat and contain darkly stained, small nuclei, with large cuboidal or columnar cells almost opposite them.

TABLE I: CALORIC UTILIZATION AT LOW BODY TEMPERATURE

State	Rabbit No.	Average rectal temperature, ° C.	Cal. per m.² per hr.	Change from basal, per cent	R. Q.
1st induction	1	20.0	109	+185	0.750
1st hour					
1st hour	2	25.0	84	+130	0.752
2nd hour		28.0	45	+27	0.765
2nd induction	3	19.0	0.68	−97	0.735
Pseudohibernating	4	19.0	0.58	−97	0.750
2nd induction	5	25.5	13	−65	0.784
2nd hour	6	25.0	15	−57	0.850
Normal control		37.0±0.5	35±2 cal.	0.0	0.800±0.050

The thyroid was of normal size and the larger vessels were engorged. Cut section revealed a grayish red, homogeneous surface. In the histological section the follicles were small and almost devoid of colloid.

There were remnants of colloid present as small islands or streaks in a few follicles; otherwise the small follicular space was filled with vacuoles. The large epithelial cells of the follicles were of uniform cuboidal shape (resembled Fig. 4 B, except for more uniformity of cell size). The capillaries were hard to find and were not engorged or dilated. The presence of large cuboidal epithelial cells and follicles almost devoid of colloid suggested an acutely hyperactive gland. Since at this low body temperature (16–21 ° C.) there is little circulation of the blood and apparently little metabolic activity, most of the hyperplasia and removal of colloid must have occurred in the earlier period at higher levels above 21° C., and the gland had little opportunity to reimburse its deficits while at the lower level.

Several animals suddenly succumbed: 1 at the end of 1 day, and 2 after 3 days, after making an apparently uneventful recovery following the prolonged hypothermic state. The thyroid gland in each instance appeared normal upon gross examination and nearly normal histologically, enough time apparently having elapsed for it to recover. The cause of death remained undetermined.

The Effect of Repeated or Greatly Prolonged Temperature Reductions—Slow Exhaustion

Nine animals were cooled and then were permitted to heat themselves to normal or almost normal temperature. Their body temperatures then were lowered again and they were allowed to recover spontaneously. In a few it was done 3 times. This is the severest strain that can be put upon the heat-regulating mechanism, and probably resembles in many respects those conditions to which the wild rabbit may be subject in its natural surroundings.

Rabbit 29 had its temperature reduced 3 times to between 26° and 29° C. over a period of 48 hours, when it succumbed.

Gross examination revealed an apparently normal thyroid gland of average size. Histological study showed many moderately dilated vessels. There was abundant colloid in most of the follicles, and in many vacuoles were present. The epithelial cells of the follicles were low cuboidal in shape. This was similar to the appearance of a normal, although rather active, thyroid.

When the animal has partially heated itself spontaneously after the first temperature reduction, each additional reduction can be obtained more rapidly and with a relatively smaller decrease in the temperature of the environment. Following each reduction, it takes the animal longer to recover. While still giving the appearance of activity, the thyroid gland in this animal was not in a hyperactive state, yet showed signs of being active, or perhaps the end results of exhaustion of a hyperactive state coincident with the final inability of the animal to increase its temperature. Thyroids of 4 animals after the second reduction showed a picture similar to that described above. Two others demonstrated a small amount of hyperactivity, and 3 definite hyperactivity without colloid, showing that variations in the state of hyperactivity occur and bear some relationship to the ability of the animals to regain their normal temperature and status. From the appearance of notable and rapidly developing hyperactivity of the thyroid following the first reduction, and similar changes in animals capable of recovering subsequent to the first hypothermic state (Fig. 4 A), one may assume that the hypertrophy could have been brought about at first and that then, as the gland or its resources became less able to supply the demands made upon it, the epithelial hypertrophy diminished (Fig. 5 A). Some of the abnormality may have been due to a decrease in chemical exchange or reduction of blood flow at the low temperature.

Animal 36 illustrates prolonged (62 hours), unsuccessful effort to recover with the thyroid gland in a hypoactive state. The period of the experiment was long enough to allow considerable change to occur. The rabbit had remained at a temperature slightly below 30° C. for 62 hours, at the end of which time it succumbed.

Postmortem examination revealed a fleshy thyroid gland approximately 1½ times the normal size, of a dull, grayish red color and rubbery consistency. Microscopic study showed an abundance of colloid. All follicles were enlarged to twice the normal size. Practically no vacuoles were present in the colloid. The epithelial cells were flattened and the nuclei large and densely stained, resembling Fig. 5 A. The gland seemed to be definitely hypoactive, and its appearance was not unlike that brought about by prolonged fasting (Fig. 5 B). Stephens (24) has noted that when rats are maintained on a subminimal diet for a period of 3 to 4 weeks, the thyroid gland assumes a notably hypoplastic state. Three control rabbits, therefore, were fasted for 3 days and then sacrificed. Their thyroid glands likewise presented a picture of hypoplasia (Fig. 5 B), which closely resembled Fig. 5 A.

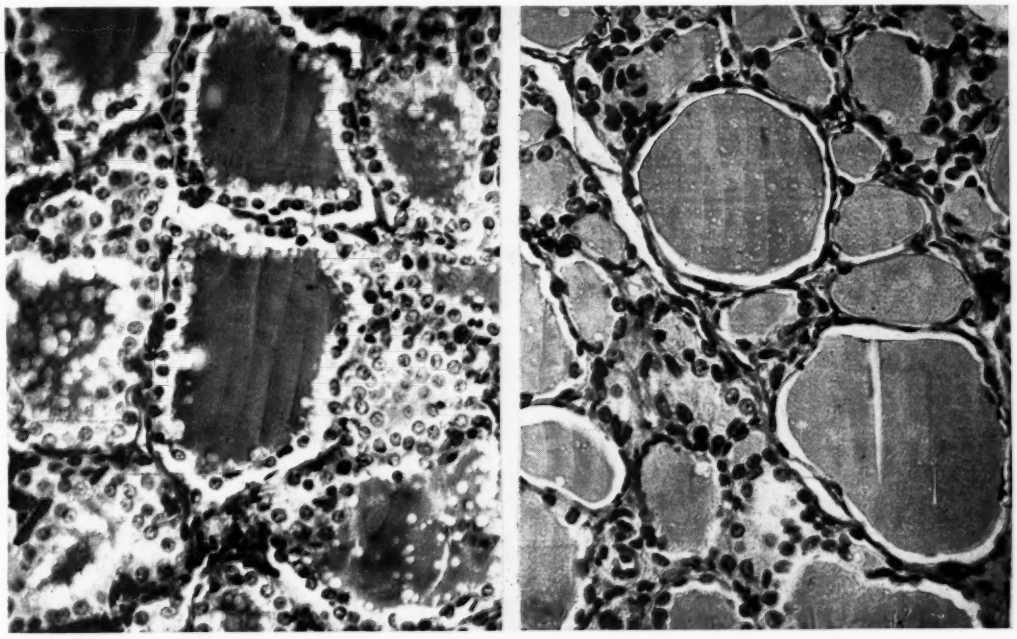
In 4 animals that could not regain their body temperature after a second reduction to the hypothermic state, the blood sugar was found to be between 20 and 40 mgm. per 100 cc. compared to a normal of 80 to 100 mgm. per 100 cc. This suggests that during attempts to regain its normal body temperature while being reduced to a hypothermic state, the animal apparently had depleted its stores of body sugar, a situation analogous to fasting for several days or a

subminimal diet for several weeks (23). Similar changes of the thyroid gland, which develop slowly and are characterized by atrophy and involution, have been described by Jackson (12), Meyers (16), and Rabinovitch (18).

Basal metabolism in hypothermia.—Basal metabolism studies were performed by Mr. Robert Ryer, III, in an attempt to correlate the morphological appearance of the thyroid gland to the oxygen consumption during various states of hypothermia. In each instance the animal was fasted for 24 hours before the metabolic studies were performed. This did not seem to change the clinical reactions to the short period of reduced body temperature in any way. The histological appearance of the thyroid glands from animals that had been fasted 1 day was essentially normal. Normal rabbits were placed in the indirect respiration apparatus, and their metabolism was calculated from the gaseous interchanges for control values. In a series of 50 New Zealand white rabbits, the average value for the normal rabbit was 35 calories per square meter per hour.

Experiment 1.—Two rabbits were cooled until their rectal temperatures were in the vicinity of 25.0° C. They were sluggishly active and yet not in the pseudohibernating state. They were placed in the respiration apparatus at room temperature, at which time they commenced to regain normal body temperature promptly. During the first hour, 1 animal with an average temperature of 25.0° C. produced 84 cal. per m.^{2} per hour, an increase of 130 per cent (Table I). During the second hour, it raised its rectal temperature to 28.0° C. and produced 45 cal. per m.^{2} per hour, an increase of 27 per cent. The other rabbit produced 109 cal. per m.^{2} per hour, an increase of 185 per cent, at an average rectal temperature of 20.0° C. Both animals recovered spontaneously.

Experiment 2.—Two rabbits were cooled to a rectal temperature of 19.0° C. at which point they were quiescent and inactive (pseudohibernating state). The



A B

FIG. 5.—Resemblance between slow exhaustion at low temperature and starvation. The rectal temperature of rabbit 34 was reduced to 17° C. in 6 hours, and the rabbit placed at room temperature. The rectal temperature rose spontaneously to 22° C. in the next 2 hours, and in the following 8 hours to 32° C., where it remained for the next 32 hours, at which time death occurred. The total elapsed time at low body temperature was 48 hours.

(A) The colloid is definitely increased in amount and stains more heavily with eosin than usual. Vacuoles are numerous. The epithelium tends to be low cuboidal, even almost flat, yet high cuboidal types are not infrequent. Many of these smaller cells appear to have shrunken away from their peripheral attachments while the larger ones remain in place.

(B) Appearance of thyroid after 3 days of fasting. The colloid is increased in amount, with areas of acini showing numerous vacuoles. The majority of the epithelial cells are flat in type, with here and there areas in which they change from flat to low cuboidal in type. The nuclei in the cuboidal cells are large and show fine details. Some sections resemble Fig. 1 A while others resemble Fig. 5 A, including the large number of vacuoles. The acini tend to be more circular than those in the controls or in the exhausted hypothermic animals.

calories produced per square meter of body surface per hour at an average temperature of 19.0° C. were 0.67 and 0.57 respectively, or the rather fantastic figure of 97 per cent below the basal metabolic rate (Table I). Both recovered spontaneously in the usual manner.

Experiment 3.—Two animals were reduced to 20.0° C. rectal temperature, and allowed to recover, following which their temperatures were lowered immediately a second time to 25.0° C. Their calory productions at this time were 13 cal. per m.^{2} per hour and 15 cal. per m.^{2} per hour, respectively, with a percentage change from the basal rate of 65 per cent and 57 per cent below the basal metabolic rate. This seems to indicate a definite depletion of reserves, yet both animals recovered spontaneously, although slowly.

SUMMARY

Profound changes in the size of the follicular epithelium and changes in the follicular contents of the thyroid gland may occur rapidly, even within one-half hour, in the various stages of artificial hypothermia. These may be extensive enough when combined with vascular engorgement to enlarge the thyroid in the gross from 1½ to 3 times its normal size. A certain amount of correlation between these histological changes and the sequence of events is possible. There is apparently at first a tremendous increase in the circulation, indicated by the increased heart rate and the engorged blood vessels in the gland. There is a progressive increase in the size of the follicular epithelium, an increase in the number of vacuoles, and a rapid diminution of colloid. Recovery from low temperatures, with histological evidence of cellular hypertrophy and decrease in the amount of colloid, may be accompanied by metabolic rates of 150 per cent above the normal. Continued low body temperatures tend to restrict the circulation and to reduce the chemical activity and exchange. Excessive demand tends to deplete the gland of its colloid, and since it is slow in being replaced the acini may be made up of large cuboidal cells and vacuoles, and yet have no ability to stimulate metabolic activity because either the supply of colloid is lacking or other mechanisms such as glucose stores may be inoperative. In a prolonged quiescent state at very low temperatures the metabolic activities may be almost abolished. If the thyroid can continue to function, even at a very slow rate, it may eventually return to normal or assume the histological appearance of slight activity, as the animal slowly recovers. If the low temperature is prolonged too much a hypotrophic status may develop, not unlike that resulting from starvation.

The mechanism for maintaining life in an artificial state of hypothermia, and the ability to recover normal body temperature following either a short or a prolonged state of hypothermia thus involve a number of complicated mechanisms calling into play several of the endocrine glands and a vast number of complex chemical reactions. The thyroid gland evidently plays an important role in this complex picture.

CONCLUSIONS

1. There is a gross enlargement of from 1 to 3 times normal size, and an engorgement of the thyroid gland, together with microscopic evidence of hyperactivity of the follicular epithelium in a large proportion of rabbits whose body temperatures have been reduced to 27–10° C. within a period of 2 to 5 hours.

2. The metabolism is greatly increased, particularly during the stage of recovery from temperatures near 25.0° C., but as the temperature falls the metabolism diminishes rapidly. It was nearly zero at the lowest temperature measured (19.0° C.).

3. If the temperature is reduced too rapidly, death occurs. An increase in circulation and follicular cell size takes place but the gland is devoid of colloid. In particularly severe reactions diffuse edema is also present in the gland.

4. Most rabbits lapse into a state of artificial hibernation at a body temperature below 28.0° C. The thyroid gland appears hyperactive, shows a loss of colloid, large cuboidal follicular cells, and a considerable increase in circulation during the early phases of this state. Yet after prolonged periods of 6 to 24 hours at this level or lower a hypoplastic status develops, usually in keeping with the length of the hibernation status, from which the animal may not be able to recover spontaneously. Recovery can be brought about in some cases by the application of heat.

5. Prolonged maintenance of hypothermia (30 to 48 hours) at higher levels of 25–28° C. may result in a thyroid picture of hypoactivity, showing excessive colloid and flattened epithelium and resembling the picture of starvation.

6. Following either partial or complete recovery from a state of hypothermia, each consecutive additional hypothermic state can be produced more rapidly and easily and with less ability on the part of the rabbit to recover. In such cases the thyroid shows an abundance of colloid, suggesting an ability of the gland to manufacture and store this material, but an inability to utilize it—probably due to the failure of other mechanisms such as glucose stores, etc.

7. The ability of the rabbit to withstand hypothermic states thus depends in part upon the state of activity of the thyroid, as well as upon its capacity to function over a prolonged period at low temperature.

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Studies on the Effect of Hypothermia

III. The Effect of a Single Short Period of Hypothermia on the Brown-Pearce Rabbit Epithelioma*

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Since hypothermia, or crymotherapy, has been advocated and is being utilized in human cancer therapy by Smith and Fay (12), it seems advisable to study the *in vivo* effect of induced hypothermia on transplantable tumors in laboratory animals under controlled conditions in order, if possible, to throw some light upon the changes brought about in such tumors by the period of reduced temperatures.

The effect of *in vitro* freezing on the takes of transplantable tumors has been studied by a number of investigators (2–6, 8–11) upon various types of growths. A pronounced variation is described in the response of different types, and even to a large extent of the same tumor. Viability also depends upon other factors, such as the way in which the tumor is frozen, *en masse* or in a saline suspension of cells; the speed of freezing, its duration (6, 8), and the number of times frozen. It appears that neoplastic cells resist intense cold suddenly applied better than do normal ones (6). Furthermore different normal tissues may vary considerably in their resistance to cold.

The reduction of body temperature to the levels of room temperature (22–26° C.) is in no way related to the conditions under which truly frozen cells have been studied. In no sense has the human patient (12, 13) or the laboratory animal been "frozen," even though ice has been used to reduce the body temperature. Thus the short experiments reported here are not comparable with the technic used for human patients, where the reduced temperature may be maintained for days. They do, however, yield evidence of a sort in this regard, since they offer data on what might occur in the early periods of prolonged exposure to low temperature, and on what effects short exposures to low temperatures may have upon the growth rate, survival period, and metastatic rate of the tumor. It should be emphasized that these data are restricted to a study of only a small part of the effects of reduced body temperature and to only three exposure or dosage levels.

METHOD

Young, susceptible, white male rabbits of the New Zealand strain were inoculated in each testicle with 0.2 cc. of a freshly prepared suspension of the Brown-Pearce rabbit epithelioma from a rapidly growing stock tumor. The resulting diffuse nodules appeared promptly and usually grew rapidly in the controls until the limits of the testicle had been reached. This time varied between 1 and 8 weeks due to variations in distribution of the suspension in the tissue. Such a procedure is not as satisfactory for precise growth studies as is the fragment method, but it was thought advisable to use a suspension in order to obtain a tumor with a minimum amount of necrosis as quickly as possible and before the animal became cachectic. In some cases, particularly in experiment 3, the growth was so rapid at first as to make the growth curves after treatment of little comparative value.

The body temperature of the rabbit was reduced in the ice bath and maintained at a given level in the refrigerator for various times. Six to 10 animals were treated at once. This technic and the physiological alterations it produced are described in another paper of this series (1). Seventeen tumor-bearing rabbits were used in the preliminary experiment to establish the technic and proper low temperature level for maintenance.

Three experiments, totaling 48 rabbits, are analyzed to observe the effect of the 6, 8, and 24 hour exposure.

In addition, 10 animals were sacrificed for histological study of the tumor at varying times up to 14 days following hypothermia. Some rabbits succumbed during treatment, or from 1 to 3 days following hypothermia, for no apparent reason, and sections of their tumors were studied microscopically. These rabbits that succumbed during or immediately following hypothermia or were sacrificed for morphological study are not included in the charts illustrating the

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growth characteristics of the tumor, nor in the data on survival time after inoculation, since it was thought that intercurrent infection or other factors not directly concerned with the tumor problem were involved. Histological study was also carried out on the primary tumors from animals that succumbed to metastasis at varying periods from 2 weeks to more than 3 months following hypothermia, to observe any morphological alteration in the tumor from the hypothermia.

DATA

Experiment 1

Twelve and 14 days after inoculation, when the tumors averaged 5.5 square cm., *i.e.* length × width (0.2 sq. cm. to 8.5 sq. cm.) 6 rabbits bearing 11 actively growing tumors were subjected to reduced body temperature of 18° C. (rectal) for 6 hours. The induction period usually consumed 2 to 4 hours and the temperature returned to normal spontaneously after removal of the animal from the cooling cabinet within 2 to 8 hours. Three of the tumors grew slowly from the start and were little affected by the treatment (Table I and Fig. 1). In 4 rapidly growing tumors, the treatment slowed the growth rate temporarily and then they grew at almost the same rate as before. Two rapidly growing tumors regressed quickly, one almost disappearing and then slowly recovering while the other continued shrinking slowly until the animal died of metastases in the third week. Two rapidly growing tumors remained almost stationary for over 2 weeks and then grew faster than before the treatment.

When the product of the lengths and widths of the tumors was averaged the average increase in size per week for the week before treatment was found to be 5.2 sq. cm.; that for the week after treatment averaged only 1.2 sq. cm., and then the average rapidity of growth increased so that by the fourth week after treatment it was 4.5 sq. cm. per week (Table I). The average length of life was 44 days (30 to 54 days) and all the animals died with widely disseminated metastases. The average length of life for stock controls was nearly 100 days, with a metastatic rate of nearly 50 per cent. The rate of growth of the stock controls was much more uniform, none of them reaching the tremendous size of the 3 treated tumors (27 to 35 sq. cm). In general both tumors in each animal responded in the same way.

Experiment 2

Fifteen rabbits with 29 tumors had their body temperatures reduced to 20° C. (rectal) for 8 hours on the fourth to the 12th days after inoculation, when the tumors were barely palpable (2 to 3 mm. in diameter) as small, diffuse masses. Nine animals of the same group with 17 tumors were kept as controls.

The growth rate of the tumors averaged 2.37 sq. cm. per week for the week previous to treatment and then the average size became smaller for 2 weeks. This value was biased by the tremendous shrinkage of 2 large tumors and the latency of 9 others during this period. Four tumors actually seemed to grow faster after treatment. The extreme spread of the growth curves, *i.e.* 6 very rapidly growing tumors and 4 slowly growing ones, was much greater than that for the controls at the seventh week, yet the averages (Fig. 3) were nearly the same for the treated and the controls (Fig. 2). In general the trend was similar except for the 2 week period after treatment (Fig. 2).

Table I: Brown-Pearce Rabbit Epithelioma

Average Change in Growth per Week in Square Centimeters

	Before treatment		After treatment								Number of rabbits
Experiment, weeks	2	1	1	2	3	4	5	6	7	8	
Control experiment 2			+0.87	0.89	1.6	0.4	0.4	0.4	2.27	0.2	9
Control experiment 3			+7.3	−1.1	+0.2	+0.9					6
Experiment 1, 6 hours at 18° C		5.2	+1.2	+1.6	3.8	4.5					6
Experiment 2, 8 hours at 20° C	0.61	2.37	−0.17	−0.17	+1.0	+1.0	1.0	0.9			15
Experiment 3, 24 hours at 30° C		6.2	+1.5	+0.25	+0.75						12

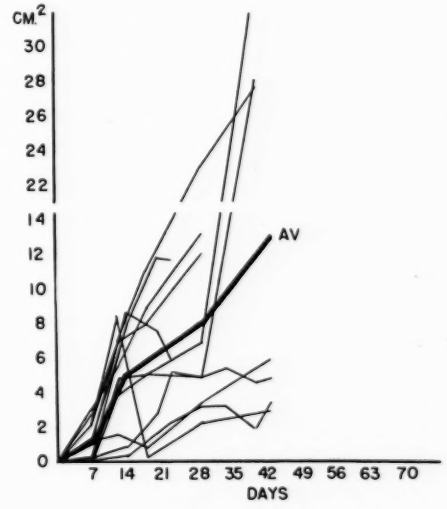


Fig. 1.—Experiment 1. Six rabbits, 11 tumors. Six hours at 18° C. between 12th and 14th days. In Figs. 1 to 6, AV = average.

EXPERIMENT 3

Twelve animals bearing 23 tumors were subjected to a state of hypothermia 3 to 5 days after inoculation. They were maintained at an average rectal temperature of 30° C. for 24 hours (Fig. 5). Their growth curves were observed for 30 days, by which time half had succumbed to metastases. Of 6 animals in the same group, bearing 12 tumors and maintained as controls, only one, or 17 per cent, died of metastases within the 30 day period (Fig. 4). This tumor grew so rapidly that the testicular structure apparently could not expand in keeping with it, and the growth curves are therefore flattened out in the second week. In 3 of the controls the tumors reached 8 to 9 sq. cm. and then shrank rapidly. Only 2 per cent of the control tumors continued to grow in the second week. This has biased the average curves, the outcome that they reproduce looking more like a treatment effect than anything seen in the treated group. This is one of the handicaps of the suspension method of inoculation.

In the treated group there were only 3 that regressed; 4 remained stationary; 6 showed no change in rate; the remainder grew somewhat less rapidly, though actively, during the week after treatment. Following this, the growth behavior was very much more erratic than that of the controls. None shrunk enough to indicate that it was going to disappear completely.

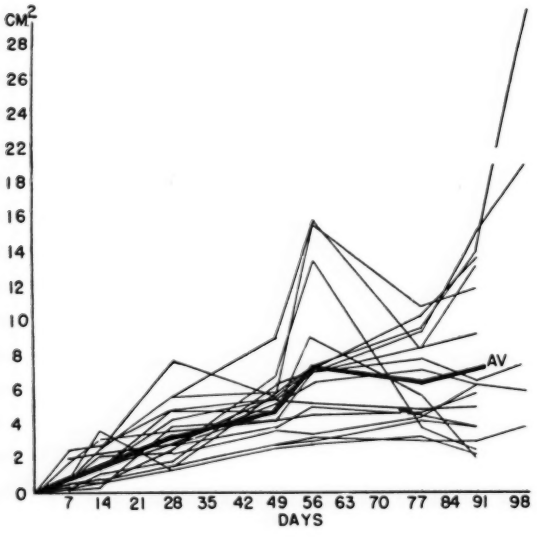


FIG. 2.—Experiment 2. Nine rabbits, 18 tumors. Controls.

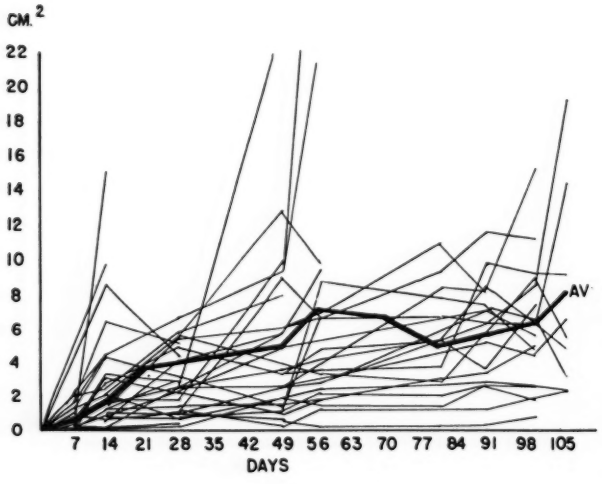


FIG. 3.—Experiment 2. Fifteen rabbits, 29 tumors. Eight hours at 20° C. between fourth and 12th days.

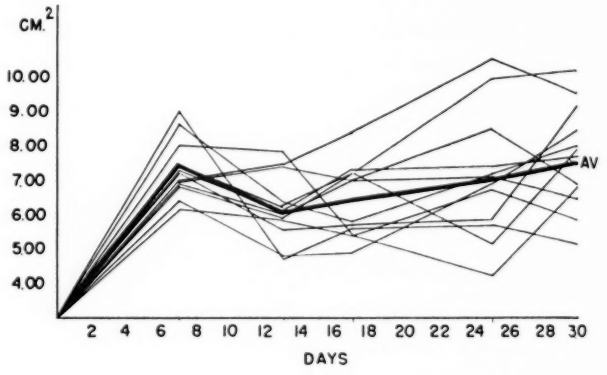


FIG. 4.—Experiment 3. Six control rabbits, 12 tumors.

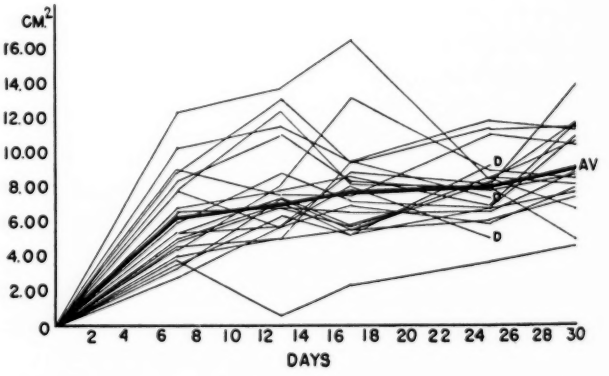


FIG. 5.—Experiment 3. Twelve rabbits, 23 tumors. 24 hours at 30° C. between third and fifth days.

The tumors in the survivors (50 per cent) at the end of 30 days resembled those in the controls. This is reflected in the parallel position of their average growth curves (Fig. 4).

There appears to be very little significant difference, as indicated by the growth curves, between the untreated and treated animals in the main growth characteristics of this tumor, after the direct effect of the treatment wears away (Fig. 6). However, the average life of the treated rabbits was consistently shorter (76 to 86 days) after inoculation, whereas the average life of the untreated tumor-bearing rabbits was well over 100 days. The usual cause of death of the treated animals was widespread metastases. The treated tumors appeared slightly larger on the whole than the untreated ones, and in fact some of the largest tumors

we have seen developed in the group receiving hypothermia. This may be due to chance, yet it seemed to occur in each experimental group.

It is difficult to determine what effect, if any, hypothermia may have upon the morphology of the Brown-Pearce rabbit epithelioma. The tumor normally presented areas of necrosis in the central portions of large rapidly growing masses as early as 5 days following inoculation, and no definite increase of necrosis in the treated tumors was observed on gross examination. Thus the abundant necrotic areas observed in sections studied after hypothermia may well have been an accompaniment of the usual growth of the tumors and in no way related to the hypothermia. A few hours after onset of the treatment the scrotum became dark and congested and autopsy at this period showed the cut tumor surface to be rather soft, red, and hemorrhagic with decided engorgement of all the superficial vessels. Specimens examined up to 5 to 7 days later sometimes contained small masses of dark red hemorrhagic tumor lying within normal looking, or slightly edematous and friable, tumor.

Microscopic examination usually revealed considerable dilatation and congestion of all vessels. Occasionally a rupture in a small vessel was seen, with the extravasation of blood cells into disorganized tumor (Fig. 7). Five to 7 days after hypothermia there were many small foci of large actively growing tumor cells arranged as a collar about the blood vessels spread throughout the extensive areas of hyalin necrosis and disintegrating tumor cells (Fig. 8). Microscopically the distribution of the necrosis suggested a zonal and partially focal disturbance related to interference with the blood supply, such as is found in old, large, regressing tumors. There was nothing resembling the cell degeneration and scarring noted after roentgen radiation.

DISCUSSION

The data presented here demonstrate that reducing the general temperature, including the tumor temperature, of rabbits for short periods of time (18° C. maintained for 6 hours, 20° C. maintained for 8 hours, and 30° C. maintained for 24 hours) has no definite lasting inhibiting effect upon the growth characteristics of the tumor.

The definite reduction in the survival period of animals treated by hypothermia, disregarding any that may succumb during or immediately following the treatment, may be related to the widespread metastases that quickly develop in treated animals (clearly in excess of the controls). This suggests that hypothermia of this duration may be instrumental in enhancing the dissemination of tumor fragments and favoring the development of metastases. We suggest that the uniform finding of diffuse hemorrhages in the treated tumors may offer the explanation for this, namely that the ruptured vessels present multiple portals of entry for dislodged cells (7).

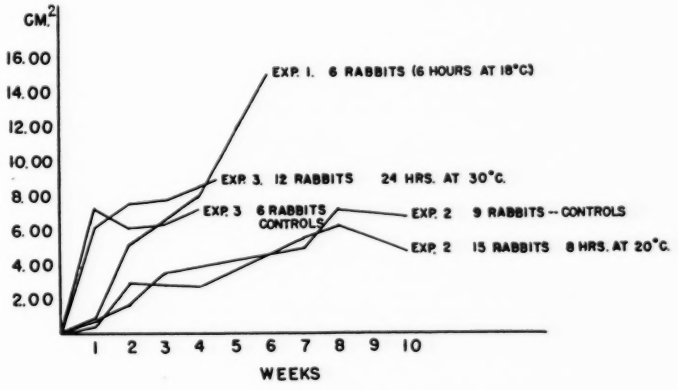


FIG. 6.—Average growth curves of controls and tumors subjected to hypothermia.

These findings differ from those described by Smith and Fay (12). They report a decrease in size of tumor during the first 24 to 48 hours of hypothermia, retardation of recurrences, diminished rate of growth during recurrences, and a definite histopathological series of events characterized by reduction of blood supply with few blood vessels discernible in section, followed a few days later by necrosis and frank disintegration of the cells, polynuclear cellular infiltration, liquefaction, and the absorption of necrotic tumor. These alterations, however, occur after prolonged exposures (5 days), and it should be emphasized that our experiments, while conducted at much lower temperatures, fell far short of such prolonged treatment and that our findings are therefore not strictly comparable.

The engorgement of the blood supply to the tumor with extensive necrosis but no prolonged inhibition

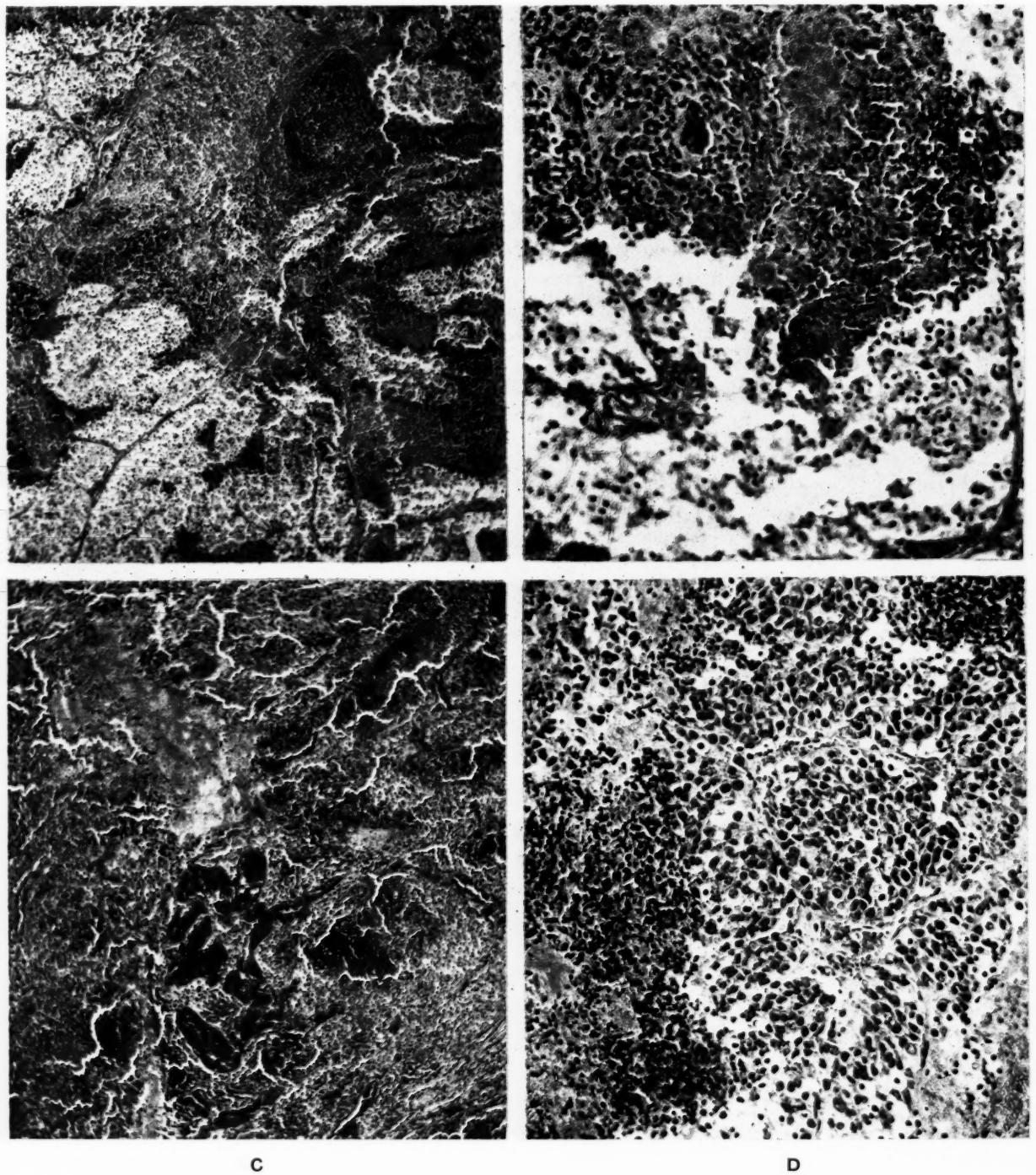


FIG. 7.—Acute changes in the Brown-Pearce rabbit epithelioma after 8 hours of hypothermia at 20° C.

The tumors were 14 days old and all were approximately 2.0 × 3.0 cm. at the time the rabbit's temperature was lowered. The immediate reaction was swelling of the tumor and darkening of the scrotum. This was followed shortly by a definite shrinking, and one week later by progressive growth in other tumors in this group (Fig. 3).

(A) Three hours after cooling; extensive edema and a few focal hemorrhagic areas in the low power view. Most of the cells are devoid of cytoplasm and show contracted dense nuclei. A few shrunken cells (the darker concentrations) near blood vessels remain intact but show little nuclear detail. Old necrosis is also visible.

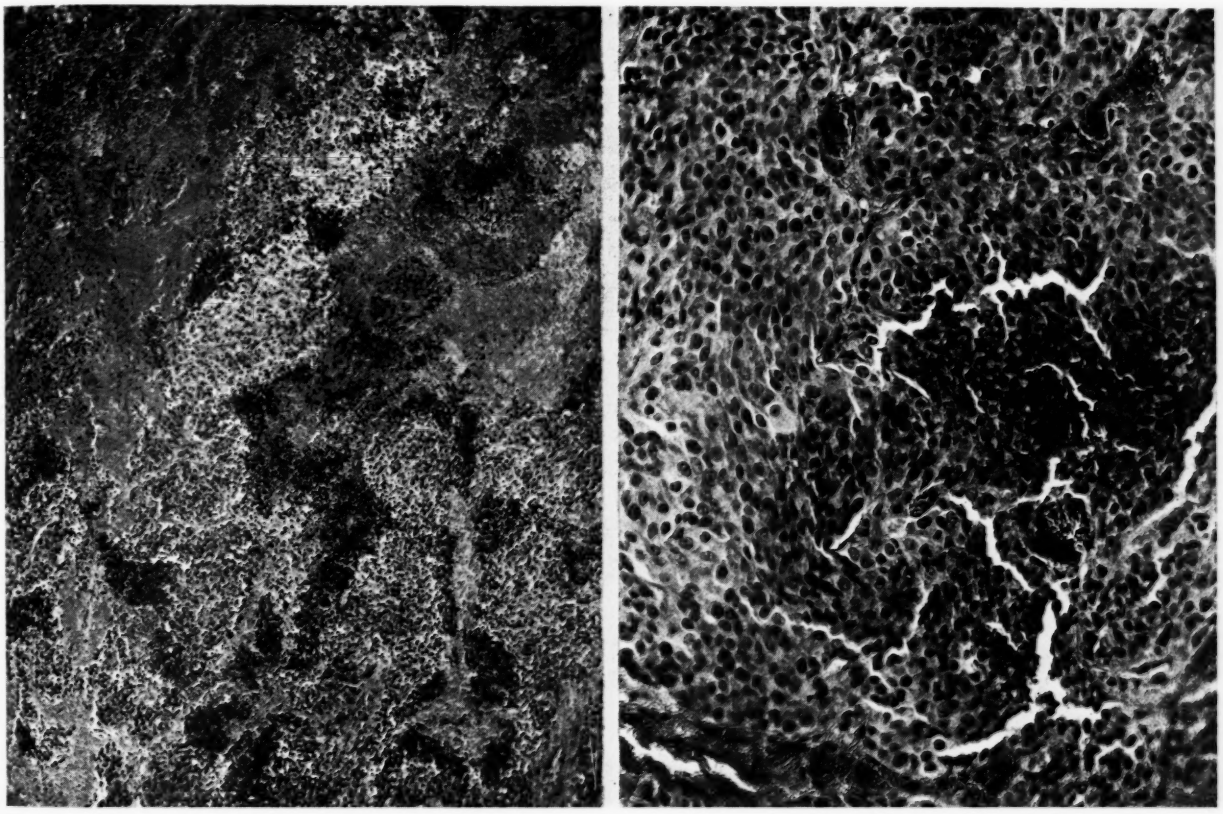
(B) High power view of one of the foci of shrunken, dense cells near small blood vessels. The periphery of the area is disrupted by edema.

(C) At 72 hours (low power view) the edema is less pronounced, the necrosis is more widespread, and the collections of dark nuclei show more clearly.

(D) In the high power view of one of these dense nuclear collections, masses of disintegrating cells are seen at the periphery of "lobules" whereas shrunken pyknotic and abnormal appearing cells are distributed around the vessels. Some of these must be viable.

(and possibly even enhancement) of its growth characteristics, together with the increased number of metastases and consequent shorter survival period, would indicate that while the short hypothermic state may have caused considerable damage to the tumor this has in no way benefited the host. While 24 hours of hypothermia is a long time in the life of the rabbit, it is probably not comparable to the 4 and 5 day period in the human subject. Great prolongation of hypothermia in the rabbit, in our experience, would be very difficult to achieve because 24 hours was found to be near the limit of endurance. In retrospect, it appears that grafts would have yielded better growth data than the suspension employed, but circumstances did not allow a repetition of our experiments. The data show a temporary delay in the growth of the tumor, and at the time we did not see any better experimental procedure for emphasizing this fact.

These experiments indicate that a certain amount of damage may be done to the tumor by this procedure, and perhaps by combining its effects with other procedures (roentgen radiation, heat, etc.) a much greater destructive effect could be obtained. Hypothermia, *per se,* enhanced rather than inhibited metastasis in the experiments here reported.



A B

FIG. 8.—Appearance of the Brown-Pearce rabbit epithelioma 7 days after 8 hours of hypothermia at 20° C.

(A) The edema, although diminished, is still prominent in some areas. Necrosis is widespread and foci of disintegrating and growing cells stand out prominently in the low power field.

(B) The large obviously growing cells may be seen around and between the small blood vessels. A small mass of dark shrunken nuclei also is visible.

CONCLUSIONS

1. Short periods of hypothermia (6 hours at 18° C., 8 hours at 20° C., 24 hours at 30° C.) caused a variety of changes in the growth curves of the Brown-Pearce rabbit epithelioma. The rate of growth was increased in a few, unchanged in approximately one-third, static in one-third, and reversed in the remainder during the week following treatment.

2. An analysis of the averages of the growth curves indicates a definite slowing of growth during the week following treatment.

3. After the second week following treatment, however, the growth rate is essentially normal (based upon the averages) although the extremes in size of tumor are greater than those for the controls.

4. The number of animals succumbing from metastases was decidedly increased and the survival period thus decreased following short periods of hypothermia.

5. Numerous tears in the vascular system of the tumor resulting from the treatment probably were responsible for the dissemination of the tumor.

6. The only definite and immediate histological alteration produced by hypothermia was a notable congestion of all vessels, with focal hemorrhage. A week later diffuse necrosis with islands of actively growing tumor cells around the blood vessels gave evidence of prompt recurrence.

7. No definite "cures" were obtained by the exposure periods and technics used.

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Studies on the Effect of Hypothermia

IV. The Rise of Serum Magnesium in Rabbits during the Hypothermic States as Shown by the Spectrochemical Method*

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In this paper are presented data demonstrating an increase in the magnesium content of the serum from 12 rabbits of a group whose body temperatures had been lowered during a test of the effect of hypothermia on the Brown-Pearce rabbit epithelioma. Within 2 to 5 hours after the start of the low temperature state (13–22° C.) the average increase in serum magnesium was 24 per cent. We believe that this bears some relation to the lethargy reported in another paper of this series.

Serum magnesium has been shown to be increased in several species of animals during normal hibernation (13, 15). It has also been demonstrated by Suomalainen (17–19) that the injection of magnesium and insulin into hedgehogs usually induces hibernation. Thus it appears that magnesium not only plays a significant role in the mechanism of normal hibernation, but also is important in artificially produced pseudohibernation (hypothermic states) and thus is worthy of some study.

METHOD

Normal, young adult, tumor-bearing rabbits of the New Zealand strain were placed in cylindrical wire cages that were immersed in melting ice and water as described in Paper I of this series. The animals were kept on a stock diet of alfalfa and oats. No urine was produced or excreted during the hypothermic state, and when the temperature of the rabbits fell below 25° C., they were in a lethargic and almost completely passive state. Alteration in serum magnesium was studied after the body temperature had been lowered rapidly from the normal 36.5–39.0° C. to 13–22° C. in a period of approximately 2 hours. Prior to immersion in ice and water, and after the body temperature had been reduced to the desired level, samples of blood of about 1.5 cc. each were taken either from an ear vein or by cardiac puncture. The blood was centrifuged with no anticoagulant added and the serum stored in an ice chest until determinations were made. Specific gravity determinations were made on several samples of serum by weighing a known amount by the method of Brown and Clark (2) to detect alteration in concentration.

Method of Magnesium Determination

The serum magnesium was determined by a spectrochemical method of analysis quite similar to that developed by Steadman (16) for serum sodium. The magnesium and sodium measurements presented here on rabbits undergoing hypothermia were made simultaneously on the same sample. It was found that the intensity of the magnesium line of 2,779.85 Å could be measured together with the sodium line of 2,680.35 Å, and that the cadmium line of 2,677.60 Å would serve as an internal standard for the magnesium as well as the sodium (16). A single determination was made on 0.25 cc. of serum diluted 1 to 40. With this was mixed 0.25 cc. of cadmium chloride solution, 1 to 4,000 cadmium as the internal standard. This was carefully ashed and the residue picked up in 0.25 cc. of 10 per cent HCl and dried in the crater of the spectrographic carbon, which was made the negative electrode of a low voltage DC arc.

The working or calibration curve of the ratio of the magnesium line intensity in the source to the cadmium line intensity against the quantity of magnesium introduced into the arc in the presence of the other serum minerals is shown in Fig. 1. The calibration curve is not appreciably affected by changes in the amounts of the other minerals, principally sodium, that occur in the present experiments. It may be observed that the curve does not pass through the origin of coordinates. This is because of the magnesium contamination of the carbons. The magnesium contamination is fairly constant in magnitude,

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however, for each lot of spectrographic carbons, and in practice pieces of carbon from the same stick were used for the measurements on each rabbit. Usually 6 determinations were made and averaged for one measurement. These may be done in about 1 hour. The average gives serum magnesium and sodium values with about ±3 per cent standard error. Somewhat different spectrochemical methods for the determination of magnesium in biological fluids have been described by Duffendack and his associates (6) and by Cassen (4).

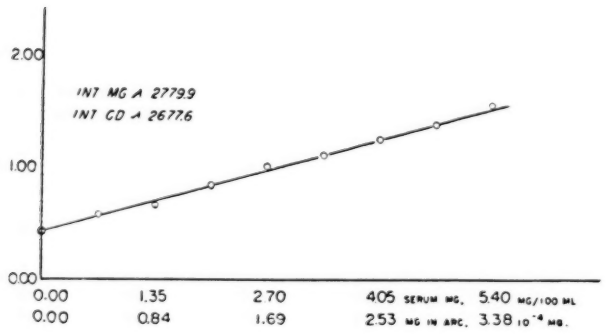

FIG. 1.—Magnesium calibration curve.

RESULTS

Table I presents the serum magnesium and sodium values in 12 rabbits whose body temperatures were reduced to various low levels (22–13° C.). "Initial serum magnesium" represents the value obtained immediately prior to the production of the hypothermic state. "Final rectal temperature" represents the deep rectal temperature level at which the sample of blood was taken to determine the magnesium during hypothermia, and "Duration of hypothermia" is the interval from beginning of the reduction of the animal's temperature to the time that the blood sample was taken for the magnesium and sodium determinations during hypothermia.

It is noted that in every case there is a definite increase in the serum magnesium. The values vary from 11 to 38 per cent above the initial level with an average of 24 per cent rise due to hypothermia. There is no apparent relationship between the magnitude of the increased magnesium concentration and either the rectal temperature or the duration of hypothermia, nor presence or absence of the tumor in these experiments.

The serum specific gravity values for rabbits 9 and 10 were 1.0287 before and 1.0285 after for No. 9, and 1.0312 before and 1.0325 after hypothermia for No. 10. These values are in the upper range of normal and not in the dehydration level. Since there is no significant change in the specific gravities in these 2 rabbits, the increase in magnesium concentration apparently is not due to loss of serum water.

TABLE I

	Hypothermia		Serum magnesium			Serum sodium		
Rabbit No.	Duration, hours	Final rectal temperature, ° C.	Initial, mgm./100 cc.	Final, mgm./100 cc.	Increase, per cent	Initial, mgm./100 cc.	Final, mgm./100 cc.	Change, per cent
1 *	5	18	2.70	3.51	30	328	369	+12
2	2	15	2.59	3.69	38	369	295	−20
3	2	15	2.43	3.24	33	352	325	−8
4	2	17	2.70	3.24	20	318	318	0
5	2	22	2.32	2.70	18	328	328	0
6	2	14	2.70	3.51	30	278	332	+19
7	2	16	2.49	3.37	35	349	345	−1
8	1	15	2.59	2.97	15	335	285	−18
9 *	3	20	2.70	3.26	21	298	335	+12
10 *	3	13	2.70	3.19	18	315	335	+7
11	3	13	2.95	3.26	11	335	335	0
12 *	3	13	2.67	3.37	26	315	321	+2
Average ...	2.5	15.9	2.63	3.27	24	327	327	+0

* Tumor-bearing rabbit.

The serum sodium changes, on the other hand, show considerable variability and do not correspond to the changes in magnesium. However, it may be noted that within the experimental error and with the exception of No. 1 and No. 8, all sodium values under the influence of hypothermia tend to approach the normal level for human serum, 330 mgm. per 100 cc. In No. 9 the serum specific gravity remained unchanged at the level of normal hydration yet the serum sodium value rose 12 per cent from a rather low normal level, while in the other animal, No. 10, which was slightly less well hydrated, the sodium values rose somewhat less (7 per cent). These two observations, and the spread in change of sodium serum values from −20 per cent to +19 per cent of the original value, suggest that the electrolyte-water balance in the hypothermic state warrants further study.

DISCUSSION

Suomalainen (17–19) noted that during the normal hibernation of hedgehogs there is a decrease of blood sugar and adrenalin, and that the liver glycogen remains constant. The injection of adrenalin into hibernating hedgehogs did not affect the magnesium, but doubled the blood sugar. Injection of magnesium caused the hedgehogs to develop a pronounced decrease in body temperature that was counteracted when calcium was injected.

Taylor and Winter (20) also observed that when magnesium was injected into patients with fever there was a drop of 1° F. in temperature with each 2 mgm. per 100 cc. increase in serum magnesium.

Hibernation may be induced in normally hibernating animals by administering magnesium and insulin (17). Hibernation in hedgehogs may be induced also by injecting insulin and placing them in a cold chamber. In the insulin hibernation, as in natural hibernation, serum magnesium increases from 40 to 60 per cent, while serum calcium shows a very slight decrease (11).

Suomalainen (17–19) has further demonstrated an increase in serum magnesium in normally sleeping hedgehogs and human subjects. Although it is apparent that magnesium is intimately linked with the phenomenon of normal hibernation, the exact mechanism of the shift of magnesium from its usual stores, mainly bone and muscle although it occurs in all the glands and cells of the body (8, 15), into the circulation, and the role that it plays in hibernation are not known.

It has been observed in this laboratory that during hypothermia rabbits become lethargic and approach a phase simulating hibernation, although rabbits do not ordinarily hibernate. Furthermore, notable hypoglycemia (blood sugar of 30 to 40 mgm. per 100 cc.) has been observed in 4 of these rabbits, associated with the increase in serum magnesium. Apparently the lethargic pseudohibernation of true homeothermic animals during hypothermia is intimately linked with increase in serum magnesium and decrease in blood sugar (3, 5, 7, 12).

In another paper of this series (1) it is shown that hypothermia first produces a decided increase in thyroid function with considerable increase in basal metabolism, followed by a definite decrease in thyroid function with a decrease in basal metabolism at times to as low as −97 per cent of normal. The increased metabolism (+130 to 185 per cent) when hypothermia is first instituted is associated with signal shivering and often voluntary muscular activity. This violent muscular activity is probably an attempt by the animal to maintain the normal body temperature and must be associated with an active carbohydrate metabolism. It has been demonstrated that magnesium plays an extensive role in carbohydrate metabolism (9, 12, 13). Schmidt and Greenberg (13) believe that it is necessary for the activity of the enzyme phosphatase. Meyerhof (10) has shown that zymohexase is catalyzed by magnesium. Cori and her associates (5) point out that magnesium functions in muscle by accelerating the conversion of glucose-1-phosphate to glucose-6-phosphate, the first step in the breakdown of glycogen to lactic acid. Scott and Packer (14) have demonstrated by means of the electron microscope that the magnesium content in muscle is very high, and almost entirely in the cell, with a small part in the tissue spaces and none in the sarcoplasm. Following active muscular stimulation there is a decrease in the muscle magnesium.

It may be that the muscle magnesium is utilized in the breakdown of carbohydrate for the production of heat, and following its utilization is accordingly shifted from the muscle into the circulation.

It is well known that an increased concentration of blood magnesium elicits soporific effects, and that in high concentrations anesthesia is produced. A mild sedative or hypnotic result follows when the serum level reaches 5 mgm. per 100 cc. At 18 to 21 mgm. per 100 cc. profound coma sets in (9, 11). Wolff (22) has found that in rabbits the corpuscles contain far more magnesium than the plasma, and that there is no parallelism between the two. The magnesium in the corpuscles is fixed, does not diffuse, and remains rather constant. The plasma level, on the other hand, undergoes the considerable variation of 2.0 to 4.0 mgm. per 100 cc. (8, 21, 22). The significance of the corpuscular magnesium content is not manifest. The lethargy observed in rabbits following hypothermia, therefore, and the decided drowsiness complained of by human beings subjected to considerable chilling may be due to increased serum magnesium, and possibly is related also to the accompanying hypoglycemia.

One might thus trace the sequence of events following the induction of hypothermia as follows: Its onset is associated with definite thyroid hyperactivity and violent muscular activity in an attempt to maintain body temperature. This is accompanied by a notably increased carbohydrate metabolism resulting in a hypoglycemia. Magnesium, a coenzyme of carbohydrate metabolism, is utilized in the metabolism of carbohydrates and shifted from its muscle and other stores into the circulation. The increased serum magnesium contributes to the lethargic state (pseudohibernation) noted in hypothermia after the shivering stops, usually below 30° C. The quiescent soporific condition of prolonged hypothermia is possibly a result of the increase in serum magnesium and a decrease in blood sugar. The serum magnesium remains at

an elevated level because of possible failure of the kidney (anuria). There is no evidence that any alterations produced by low temperature in the testicular tumors (2 to 5 cc. volume) of 4 rabbits analyzed in this series contributed in any way to the changes noted in either the magnesium or sodium levels. The initial values in tumor-bearing rabbits, however, were all within normal limits for their species.

CONCLUSIONS

1. A spectrochemical method was used to determine serum magnesium and serum sodium levels at the beginning and after 2 to 5 hours of the hypothermic state in 8 normal and 4 tumor-bearing rabbits.

2. The initial serum magnesium level averaged 2.63 mgm. per 100 cc. (2.32 to 2.95 mgm.)

3. The serum magnesium showed a rise of 24 per cent (11 per cent to 38 per cent) above the initial values after the animals had been from 2 to 5 hours in the hypothermic state.

4. There did not seem to be any relationship between serum magnesium levels or their change and the duration of the low temperature (2 to 5 hours) nor its level (13–22° C.), nor to the serum sodium values, nor to the serum specific gravity in 2 animals.

5. From other data reported elsewhere in this series, there did seem to be a relation between the elevation of the serum magnesium and the depressed thyroid activity, hypoglycemia, low oxygen consumption, and the lethargic state during hypothermia. This relationship is not entirely clear and warrants further study.

6. The cessation of kidney function may have prevented the excretion of magnesium, or the cold state may have prevented its return to its storage sites.

7. There is no clear explanation of the wide fluctuations in the serum sodium (-20 per cent to $+19$ per cent) found in the hypothermic states in these 12 rabbits.

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American Association for Cancer Research, Inc.

Special Meeting of the Board of Directors

February 12, 1943

A special meeting of the Board of Directors was held at the office of the American Society for the Control of Cancer, Inc., 350 Madison Avenue, New York City, on February 12, 1943.

The meeting was called by order of the President, Shields Warren, by notices sent to all Directors from the office of the Acting Secretary-Treasurer at New Haven, Connecticut, and dated the thirtieth day of January, 1943.

The meeting was called to order at 2 p. m. by the President. Directors Halsey J. Bagg, James B. Murphy, Shields Warren, George M. Smith, Carl Voegtlin, and William H. Woglom were present. Director C. C. Little was represented by proxy.

Reading of the minutes of regular meetings of the Board of Directors held in Boston, March 30 and April 1, 1942, was waived, at the suggestion of the President, by unanimous action.

Reports of officers and standing and special committees also were waived.

NEW BUSINESS

Transfer of Custodianship of Funds

It was unanimously

RESOLVED, that the Greenwich Savings Bank of 1356 Broadway and 985 Sixth Avenue, in the Borough of Manhattan, City of New York, be hereby designated as a depository of funds of the American Association for Cancer Research, Inc., up to $7,500.00, and be authorized to honor drafts of the Association signed by its President or its Secretary-Treasurer until this authority shall be revoked and written notice of the revocation of authority of the officers herein mentioned shall be received by said Greenwich Savings Bank; and further

RESOLVED, that the Secretary furnish said Bank with a certified copy of these resolutions.

The action of the Secretary-Treasurer in the transfer of other funds of the Association from Buffalo to the Union and New Haven Trust Company, at New Haven, Connecticut, was approved.

Action Pertaining to Members

The resignations of the following three members were presented and accepted.

Beard, Howard H., Ph.D., Louisiana State University, New Orleans, La.
Berrill, Norman J., Ph.D., Sc.D., Department of Zoology, McGill University, Montreal, Canada.
Kraus, Ezra J., Ph.D., University of Chicago, Chicago, Ill.

The Secretary presented the names of four members whose deaths had been made known to the office of the Association during the past year. The Association regrets to announce their loss.

Allen, Edgar, Ph.D., Sc.D., Yale University School of Medicine, 333 Cedar St., New Haven, Conn.
Crile, George W., Ph.D., M.D., 2620 Derbyshire Rd., Cleveland Heights, Ohio.
Edwards, Harold G. F., M.D., 417 Medical Arts Building, Shreveport, La.
Ill, Edgar A., M.D., 1004 Broad St., Newark, N. J.

The nominations of nine applicants for membership in the Association were presented, and all were duly elected to membership.

Auger, Carlton, M.D., Laval University, Quebec, Canada.
Coman, Dale R., M.D., 3715 Chestnut St., Philadelphia, Pa.
Dougherty, Thomas F., Ph.D., Yale University School of Medicine, 333 Cedar St., New Haven, Conn.
Evans, Titus Carr, M.S., Ph.D., College of Physicians and Surgeons, 630 W. 168th St., New York, N. Y.
Flory, Curtis M., M.D., Ph.D., Cornell University Medical School, 1300 York Ave., New York, N. Y.
Guzman, Leonardo, M.D., National Radiation Institute, Santiago, Chile.
Schlenk, Fritz, Ph.D., M. D. Anderson Hospital for Cancer Research, University of Texas, Houston, Texas.
Shrigley, Edward W., Ph.D., M.D., Yale University School of Medicine, 333 Cedar St., New Haven, Conn.
Toth, Benedict J., M.D., 1907 W. State St., Olean, N. Y.

It was duly voted that members in foreign or occupied countries or territories be transferred to an inactive list subject to reinstatement as active members after the war.

It was duly moved, seconded, and voted that the Secretary remove from the List of Members the names of those whose annual dues have been unpaid for three years, provided that 15 days prior to such action the delinquent member has been notified of the necessity for such action and, in the interim, has not expressed a desire to retain membership. This does not apply to members who may be exempt from dues because of active service with the armed forces.

Cancer Research

The action of the Directors and members of the Association at the last annual meeting on the contribution voted to the journal, *Cancer Research,* was clarified. The obligation of the Association in this connection was assumed January 1, 1943. The extent of this obligation shall be $1.00 each year for each active member in good standing. In view of the decreased expenses anticipated for 1943 due to postponement of the annual meeting it was proposed, seconded, and voted that the Acting Secretary-Treasurer be authorized to pay to *Cancer Research,* in addition to

$1.00 per member, an amount for this one year, January 1, 1943, to December 31, 1943, such that the total of $500.00 will be contributed.

The motion was duly made, seconded, and voted that the Secretary request Dr. C. C. Little, *Chairman of the Journal Committee,* to draw up a resolution formally expressing the appreciation of the support of the journal, *Cancer Research,* afforded by grants from several cancer research foundations, and expressing the desire of the Association to assume eventually a greater responsibility in the support of the journal.

The resolution is as follows:—

WHEREAS the support given to the journal, *Cancer Research,* by The International Cancer Research Foundation, The Jane Coffin Childs Memorial Fund for Medical Research, and The Anna Fuller Fund is an outstanding and vital contribution to cancer research at a time when that support is sorely needed; and

WHEREAS the American Association for Cancer Research, Inc., represents the largest national body representative of those research workers who benefit by this generosity; be it

RESOLVED, that the American Association for Cancer Research, Inc., place on the minutes an expression of lasting gratitude for the service thus rendered; and further be it

RESOLVED, that it is the desire and intention of this Association to assume as soon as possible and to the greatest extent possible an increasing share of responsibility for the maintenance of the journal, *Cancer Research.*

Annual Meeting

No plans for the next annual meeting of the members were made other than that the meeting be held in connection with the Federation of American Societies for Experimental Biology.

Publications

The Acting Secretary-Treasurer, by unanimous action of the Directors, was instructed to submit for publication in *Cancer Research* the Proceedings of this meeting, the amended By-Laws, and the revised List of Members, designating those in active service with the armed forces, and to pay expenses incurred by such publications.

Amendments

It was duly voted that the By-Laws be amended by adding a subsection to be designated (h) under Article I, *Section 1,* reading as follows: "(h) Emeritus Members—who have attained the age of 65 years, have been members for ten years or more, and who upon application may be exempt from dues." This adds to the By-Laws an action taken by the Directors and reported to the members at a previous meeting.

Executive Committee

Exercising the authority invested in him by the By-Laws, Article II, *Section 1* (d), President Shields Warren appointed an Executive Committee consisting of Dr. James B. Murphy, *Chairman;* Dr. George M. Smith; and Dr. William U. Gardner, to act during the present emergency, when a quorum of Directors cannot be obtained and, in such case, to exercise all the authority invested in the Board of Directors.

At the request of the Acting Secretary a statement of appreciation of the efforts of Dr. William H. Woglom was made for editing the Proceedings of the Scientific Sessions of the Boston Meetings.

It was suggested that efforts be made to obtain an abstract for every paper to be presented at future Scientific Sessions, uniform as to style, length, and so forth.

Meeting adjourned at 4:20 p. m.

Shields Warren, *President*
W. U. Gardner, *Acting Secretary-Treasurer*

American Association for Cancer Research, Inc.

By-Laws

Article I

Section 1. Members. There shall be eight classes of members as follows:

(a) Donors—who shall give to the Corporation the sum of $1,000 or more each. Donors shall be members for life without dues.

(b) Life Members—who shall give to the Corporation the sum of $100. Life members shall be members for life without dues.

(c) Patrons—who shall pay dues to the Corporation of $50 per annum.

(d) Sustaining Members—who shall pay dues to the Corporation of $25 per annum.

(e) Contributing Members—who shall pay dues to the Corporation of $10 per annum.

(f) Active Members—who shall pay dues to the Corporation of $3.00 per annum.

(g) Honorary Members—who need not pay dues to the Corporation.

(h) Emeritus Members—who have attained the age of 65 years, have been members for ten years or more, and who upon application may be exempt from dues.

All persons who, on the date of incorporation of the Corporation, were active members in good standing of the American Association for Cancer Research, an unincorporated association, shall become Active Members of the Corporation as of such date.

All persons who, on the date of incorporation of the Corporation, were Honorary Members of said American Association for Cancer Research shall become Honorary Members of the Corporation as of such date, without payment of dues.

Donors, Life Members, Patrons, Sustaining Members, Contributing Members, and Active Members shall have equal privileges of membership and each shall be entitled to one vote at all meetings of members.

Section 2. Election of Members. The Board of Directors at any time, and from time to time, may elect to membership such persons as they consider eligible.

Section 3. Meetings of Members.

(a) The annual meeting of members shall be held at such time and place, within or without the State of New York, as may be designated in the notice of the meeting.

(b) Special meetings of members may be called by the President or, in his absence or disability, by the Vice-President, only upon (a) the request of the Board of Directors or (b) the request of thirty or more members. Special meetings shall be held at such time and place within or without the State of New York as may be designated in the notice of the meeting.

(c) Notice of each meeting of members, whether annual or special, shall be printed or written, signed by an executive officer, and mailed to each member at his address as it appears upon the books or records of the Corporation not less than ten days nor more than forty days prior to the date of the meeting, and the notice of each special meeting shall state the purpose or purposes for which it is to be held.

(d) A quorum of members shall consist of not less than one-third of the total number of members; provided, however, that if there shall be twenty-seven or more members of the Corporation, a quorum shall consist of nine.

Section 4. Resignations. Resignations of members shall be in writing addressed to the Board of Directors, but no resignation shall be accepted or take effect if the member presenting it shall be indebted to the Corporation.

Section 5. Expulsion. Any member who fails or neglects to pay his dues, or whose conduct shall in the opinion of the Board of Directors be prejudicial to the welfare of the Corporation, may be dropped from membership by the Board of Directors; provided, however, that notice in writing shall be given to such member of the action contemplated and of the grounds therefor at least 15 days previous to the date for the proposed expulsion of such member, and that such member shall be given an opportunity to present in written form and/or by personal appearance before the Board of Directors a defense of the act or acts upon which the proposed expulsion is based.

Section 6. Transfers of Membership. Memberships may be transferred by the Executive Committee from one class to another because of changes in the sum paid annually.

Article II

Section 1. The Board of Directors. The number of directors shall be twelve, who shall be members and who shall divide themselves into three classes. The first class shall serve until the first annual meeting of members, the second class shall serve until the second annual meeting of members, and the third class shall serve until the third annual meeting of members. Directors shall be elected at each annual meeting of members to succeed the directors whose terms shall have expired, and each director then chosen shall serve for a term of three years and until the election of his successor.

Section 2. Duties of Board of Directors. The Board of Directors shall have the following powers and duties:

(a) To nominate candidates for President, Vice-President, and Secretary-Treasurer of the Corporation.

(b) To fill vacancies that may from time to time occur among the officers.

(c) To direct and supervise the activities of the Corporation, its representatives, and employees.

(d) To appoint such committees, standing and special, as may be necessary or desirable to carry on the work of the Corporation.

(e) To prepare or cause to be prepared and to approve annually a budget for the ensuing fiscal year.

(f) To supervise, either directly or through a duly appointed committee, the investment and disbursement of all funds of the Corporation.

(g) To fix the compensation of all representatives and employees of the Corporation.

(h) To present at the annual meeting of members a report as provided by Section 46 of the Membership Corporations Law.

(i) To elect such other officers as may be necessary properly to carry on the work of the Corporation.

(j) To perform such other duties as may be required by law.

Section 3. Annual Meetings. The Board of Directors shall meet immediately after each annual meeting of members in such place, within or without the State of New York, as may be designated in the notice of the meeting.

Section 4. Special Meetings. Special meetings of the Board of Directors may be called by the President or, in his absence or disability, by the Vice-President, and shall be called by him at the request of any two members of the Board of Directors. Special meetings may be held at such time or place, within or without the State of New York, as may be designated in the notice of the meeting. Notice of special meetings shall be given by mail, telephone, telegram, or personally, and shall be so given at least four days before the date of the meeting.

Section 5. Quorum. A quorum of the Board of Directors shall consist of five of its members.

Section 6. Vacancies. Any vacancy or vacancies in the Board of Directors however caused may be filled by a majority of the remaining members of the Board of Directors then in office, though a majority of such remaining members do not constitute a quorum. The director chosen to fill such a vacancy shall hold office only for the unexpired portion, if any, of the term of the director whose office shall have become vacant.

Article III

Section 1. Officers. The officers of the Corporation shall be a President, a Vice-President, and a Secretary-Treasurer, selected from the Board of Directors. The officers shall be elected by the members either from the candidates nominated by the Board of Directors or from candidates nominated by the members. Each officer shall hold office until the next annual meeting of the members and until his successor shall have been elected and shall have qualified.

The Board of Directors may appoint other officers, including an additional Vice-President and an Assistant Secretary-Treasurer to serve for such terms and perform such duties as the Board of Directors may prescribe.

Section 2. Duties of Officers.

President. The President shall be the chief executive of the Corporation and shall preside at all meetings of the Board of Directors.

Vice-President. The Vice-President, in the absence or during the disability of the President, shall have all the powers and perform all the duties of the President.

Secretary-Treasurer. The Secretary-Treasurer shall have the care and custody of all funds and securities and valuable documents belonging to the Corporation. The Secretary-Treasurer shall deposit the funds of the Corporation to its credit in such banks, trust companies, or depositaries as the Board of Directors, or the Committee appointed by the Board of Directors to supervise the investment and disbursement of the funds of the Corporation, may designate. The Secretary-Treasurer shall sign all checks and drafts in the name of the Corporation. The Secretary-Treasurer shall keep full books of account of the Corporation and shall render to the Board of Directors from time to time at its request, and annually to the members, a full statement in regard to its funds.

The Secretary-Treasurer shall keep all minutes and proceedings of the members and of the Board of Directors in books kept for that purpose. He shall give notice of all meetings of the members and of the Board of Directors. He shall have the custody of the seal of the Corporation and shall affix the same when authorized to do so by the Board of Directors.

Article IV

General

Section 1. Fiscal Year. The fiscal year of the Corporation shall begin on the first day of January in each year and shall end on the following 31st day of December.

Section 2. Dues. Dues shall be payable in advance on or before the first day of March, in each year.

Section 3. Local Work. Branches of the Corporation may be formed in the name of the Corporation in such areas as may be approved by the Board of Directors, to carry on work in conjunction with the Corporation under the direction and subject to the approval of the Board of Directors of the Corporation.

Section 4. Seal. The seal of the Corporation shall be circular in form with the words, "American Association for Cancer Research, Inc." on the circumference and the following words in the center:

Corporate Seal 1940 New York.

Section 5. Order of Business. At all regular meetings of members and of the Board of Directors, the order of business shall be as follows:

1. Roll call.
2. Reading of minutes.
3. Reports of officers.
4. Reports of standing committees.
5. Reports of special committees.
6. Reports of other representatives of the Corporation.
7. Unfinished business.
8. New business.
9. Adjournment.

At every annual meeting of members, the Directors, after the reading of the minutes, shall submit a report as required by Section 46 of the Membership Corporations Law.

Section 6. Execution of Instruments. All formal agreements shall be signed by the President or the Vice-President or the Secretary-Treasurer unless otherwise ordered by the Board of Directors.

Article V

Section 1. Amendments to By-Laws. These By-Laws may be amended, added to, or repealed without notice at any annual meeting or with notice at any special meeting of members by a two-thirds vote of the members present or represented by proxy, or without notice at any annual meeting or with notice at any special meeting of the Board of Directors by a two-thirds vote of the Directors present, as the case may be. Any amendment made by the Board of Directors shall be reported to the members at the next annual meeting.

American Association for Cancer Research, Inc.

List of Members*

1943

A

ABELS, JULES C., M.D., Memorial Hospital, York Ave. at 68th St., New York, N. Y.
ACKERMAN, LAUREN V., M.D., Ellis Fischel State Cancer Hospital, Highway 40 and N. Garth Ave., Columbia, Mo.
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ALGIRE, GLENN HORNER, M.D., National Cancer Institute, Bethesda, Md.
AMOLSCH, ARTHUR LEWIS, M.D., Wayne University College of Medicine, 1512 Antoine St., Detroit, Mich.
ANDERSON, RUDOLPH J., PH.D., 101 Cottage St., New Haven, Conn.
ANDERVONT, HOWARD B., SC.D., National Cancer Institute, Bethesda, Md.
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AUB, JOSEPH C., M.D., Massachusetts General Hospital, Boston, Mass.
AUGER, CARLETON, M.D., Laval University, Quebec, Canada.

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BROWN, SAMUEL, M.D., University of Cincinnati, 707 Race St., Cincinnati, Ohio.
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BURROWS, MONTROSE T., M.D., 201 N. El Molino Ave., Pasadena, Calif.

C

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CHILDS, S. WINSTON, JR., 1 Wall St., New York, N. Y.
CHILDS, STARLING W., 1 Wall St., New York, N. Y.
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CLAWSON, BENJAMIN J., M.D., PH.D., 500 S. E. Delaware St., Minneapolis, Minn.
CLINE, JOSEPH K., PH.D., University of Texas, Medical Branch, Galveston, Texas.
CLOUDMAN, ARTHUR M., PH.D., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

* In the armed forces.

Clowes, George H. A., Ph.D., Sc.D., Eli Lilly and Company, Indianapolis, Ind.
Coca, Arthur F., M.D., 425 Grant Ave., Oradell, N. J.
Cohen, Mortimer, M.D., 5615 Bartlett St., Pittsburgh, Pa.
Cohen, Philip P., Ph.D., M.D., Service Memorial Institute, University of Wisconsin Medical School, Madison, Wis.
* Cole, R. K., Ph.D., Cornell University, Ithaca, N. Y.
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Abstracts

Experimental Research, Animal Tumors

Hepatic Vitamin A in the Rat as Affected by the Administration of Dibenzanthracene. Abels, J. C., Gorham, A. T., Eberlin, S. L., Halter, R., and Rhoads, C. P. [*Memorial Hosp., New York, N. Y.*] J. Exper. Med., **76**:143-161. 1942.

The administration of the carcinogen, dibenzanthracene, to rats resulted in a decrease of the concentration of vitamin A in the livers of the animals. This decrease did not seem to be due to a general hepatic insufficiency caused by the carcinogen, for the many liver tests carried out indicated that the livers were normal. The authors suggest that the dibenzanthracene has a particular effect on the ability of the liver to store vitamin A and that this effect may be due to a competition between vitamin A and dibenzanthracene for some substance, possibly a protein, to which vitamin A may be bound in the liver.—D. S.

Induction of Hepatic Lesions, Hepatomas, Pulmonary Tumors, and Hemangio-Endotheliomas in Mice with o-Aminoazotoluene. Andervont, H. B., Grady, H. G., and Edwards, J. E. [*Nat. Cancer Inst., Bethesda, Md.*] J. Nat. Cancer Inst., **3**:131-153. 1942.

Subcutaneous injections of *o*-aminoazotoluene moistened with glycerol were given to mice of strains A, C3H, C, C57 black, and hybrids derived from strains A, C3H, and C.

The compound induced hepatic lesions and hepatomas in all inbred strains and in the hybrids. The females of all groups were far more susceptible to induced macroscopic hepatic lesions and hepatomas than were the males.

The compound also induced pulmonary tumors in mice of strains A and C and their hybrids. These tumors were indistinguishable microscopically from those arising spontaneously or induced by other carcinogens. The inheritance of susceptibility to the *o*-aminoazotoluene-induced pulmonary tumors was similar to that of spontaneous or hydrocarbon-induced pulmonary growths.

Hemangioendotheliomas were induced in all inbred strains and in certain hybrid groups. These tumors involved a variety of sites remote from the site of administration.

Thus the compound moistened with glycerol is capable of eliciting tumors in tissues distant from the site of subcutaneous injection, and its carcinogenicity is not restricted to hepatic tissues.—F. L. H.

Epithelial Tumours of the Bladder in Dogs Induced by Pure β-Naphthylamine. Bonser, G. M. [*Univ. of Leeds, England*] J. Path. & Bact., **55**:1-6. 1943.

β-Naphthylamine purified by distillation *in vacuo* and crystallization from petroleum ether, was given by mouth to dogs (3 male, 1 female) during 1 to 5 years. The initial dose (150 mgm. daily) produced hematuria; the amount was then lowered to 100 mgm. and raised later to 700 mgm. One dog (male) died after 1 year without evidence of neoplastic change in the bladder. The remaining 3 died or were killed after 3 years and 8 months to 5 years. All showed papillomatosis of the bladder, but the ureters, renal pelves, and urethra were free from tumors and no metastases were found. The bladder epithelium showed a gradation of changes from simple hyperplasia to carcinoma with lymphatic permeation and invasion of smooth muscle. There were: (a) simple transitional cell papillomas, and (b) malignant papillomas, composed of transitional or anaplastic polygonal cells, or of tubular structure, and any one malignant tumor might show a mixture of these types. Permeation of perivascular lymphatics and a nodule of anaplastic carcinoma lying deep in the bladder musculature were observed. The dogs that lived longest showed most invasive growth. The dog appears to be more susceptible to these tumors than to those induced in the skin by tar. The question whether β-naphthylamine or one of its metabolites is the carcinogenic agent remains open.—E. L. K.

Lung Tumours in Mice and Man. Campbell, J. A. [*Nat. Inst. for Med. Research, London, England*] Brit. M. J., **1**:179-183. 1943.

A discussion and retabulation of data given in nine earlier papers of which the last three have been abstracted in this journal (*Cancer Research,* **1**:328. 1941; **2**:579. 1942; **3**:417. 1943) and a comparison of the data obtained from mice with those available for the incidence of cancer of the lung in man. The significance of the increase in the incidence of primary lung cancer in mice exposed to various dusts is classified as follows:—highly significant: tarred road dust, $Al_2O_3+SiO_2+Fe_2O_3+CaCO_3$; definitely significant: Fe_2O_3, Czechoslovak pitchblende dust containing Ra and As, tar-free and tarred road dust; significance near border line: diluted "nickel" dust, Czechoslovak pitchblende dust with no As and practically no Ra, bituminous coal; increase possibly not significant: $SiO_2+Fe_2O_3$, SiO_2+"nickel" dust, carbon exhaust soot, $Al_2O_3+SiO_2+Fe_2O_3$; no significant increase: steel grindings, anthracite coal, coal soot.

The incidence of lung tumors upon 475 mice exposed to dusts containing SiO_2 or Fe_2O_3 or both was 20.1% (42 simple and 50 malignant tumors), and upon 346 control mice was 7.8% (16 simple and 11 malignant tumors). The author considers that "so far as many of

the causes of the increase in incidence of lung tumors are concerned mice and man show very fair agreement." The author finds it possible to distinguish by microscopic examination 8 arbitrary degrees of deposition of dust in the lungs and tracheobronchial lymph nodes. Exposure to any of the dusts employed increases the lymphoid tissue in the lungs and lymph nodes. "When iron was present many of the 'lymph' cells in the lung appeared to be of a sarcomatous type and tended to spread out into the lung tissue; this is apparently a chemical effect." There is no regular relationship between the amount of dust deposited in these tissues and the incidence of lung tumors.

Twelve photomicrographs of lung tumors and metastases (oat cell, spindle cell, adenocarcinoma, papilloma) from mice exposed to various dusts are given. Eighteen cases of "extensions into other organs have now been obtained among the experimental mice and only two such cases among the control groups. The most common extension is into the tracheobronchial lymph nodes, but extensions have been observed also in the heart, chest wall, and kidney. The tumours seem to arise often in collapsed areas of the lung, usually near the pleura; the cells become more or less cubical, and it is not possible to decide whether these cells arise from cells of the alveoli or as extensions from the terminal bronchioles. Many types of malignant cells may be found in one and the same mouse tumour, with transitions from the usual cells seen in adenocarcinoma to spindle-like cells or the larger and smaller oat cells, or to sarcoma-like cells." The author does not describe the range of structure seen in the tumors of control mice. Tumors arising from the larger bronchi are more common in man than in the mouse.—E. L. K.

Induction of the Carbon Tetrachloride Hepatoma in Strain L Mice. Edwards, J. E., Heston, W. E., and Dalton, A. J. [*Nat. Cancer Inst., Bethesda, Md.*] J. Nat. Cancer Inst., **3**:297-301. 1942.

Earlier communications on the induction of carbon tetrachloride hepatomas in mice reported that this tumor had been obtained frequently in C3H strain mice, which have a high incidence of spontaneous hepatomas as well as in A, Y, and C strain mice, which develop relatively few spontaneous hepatomas. In this paper results are given on the induction of hepatomas by carbon tetrachloride in mice of L strain, another strain with a low incidence (1.3%) of spontaneous tumor of this type. To 73 mice, 0.1 cc. of a 40% solution of carbon tetrachloride in olive oil was administered by stomach tube 3 or 2 times weekly. The resulting hepatoma incidence was 46.6%. Histologically, the induced tumors were similar to the carbon tetrachloride hepatomas of other strains already described.—G. W. W.

Quantitative Studies on Tumor Production in Mice by Benzpyrene. Gottschalk, R. G. [*Univ. of Liége, Liége, Belgium*] Proc. Soc. Exper. Biol. & Med., **50**:369-373. 1942.

Twenty mice (University of Liége albino stock) in each of 8 groups received a single subcutaneous injection of 3,4-benzpyrene dissolved in 0.5 cc. neutral olive oil. The dose per mouse in each group varied by geometric progression from $4\times10^{3}\gamma$ to $4\times10^{-2}\gamma$ of the hydrocarbon. The oil alone was negative on repeated injection. Four gamma was the smallest amount of benzpyrene to produce a tumor under these conditions; 0.4γ and 0.04γ were not effective. The percentage of tumors obtained was greater with the larger amounts of carcinogen. Also there was a definite increase in the latent period with decrease of the amount of carcinogen injected.

The role of volume in carcinogenesis was studied by using the same amount of benzpyrene (400γ), and varying the amount of olive oil from 0.015 cc. to 1 cc. Twenty mice were used to test each volume. When the solvent was 0.125 cc. or more, at least 40% of tumors were produced; when the solvent was 0.0625 cc. or less, the percentage of tumors was below 25. The corresponding average latent periods were without relation to the volume injected.

The rate of absorption of benzpyrene and oil was studied by aspirating the oil from the cysts, after locating it by its ultraviolet fluorescence, and then determining spectrographically the amount of benzpyrene in each cyst. The amount of benzpyrene identified was lower than expected from the weight of the remaining oil. The rate of oil absorption was irregular and without relation to the amount of benzpyrene injected or to the time of appearance of the tumor.—M. B.

Fluorescence Studies of Carcinogens in Skin. I. Histological Localization of 20-Methylcholanthrene in Mouse Skin after a Single Application. Simpson, W. L., and Cramer, W. [*Barnard Free Skin and Cancer Hosp., St. Louis, Mo.*] Cancer Research, **3**:362-369. 1943.

Studies were made of the fluorescence in near ultraviolet light of frozen sections of normal mouse skin and of skin from approximately 40 Swiss mice at intervals after the application of a benzene solution of methylcholanthrene. Routine macroscopic examinations were made in ultraviolet light of more than 100 Swiss mice to determine gross changes in fluorescence after painting with the carcinogen.

Absorption of the carcinogen was found to be selective and extremely rapid. Within 2 minutes the yellow-green fluorescence of dry methylcholanthrene was present on the surface, and the blue-violet fluorescence of dissolved methylcholanthrene was confined to sebaceous glands, free lipids of the keratinized epithelium, and a variable number of fat cells of the subcutaneous fatty layer. Changes in the fluorescence and in stained control sections showed that a rapid degeneration of the sebaceous glands occurred during the first to third days accompanied by an outpouring of the strongly fluorescent sebum through the hair follicles and onto the surface of the skin. At the same time a thickening of the epidermis and epithelium of the hair follicles occurred. With the outflow of sebum the surface crust of dry carcinogen was redissolved to produce a thicker layer of brilliantly fluorescent sebum throughout the keratin. After a period varying from 4 to 8 days the keratin layer flaked off, carrying with it the blue-violet fluorescent sebum and also the hairs that had been pushed out of the follicles as the hyperplasia of the follicular epithelium occurred. Penetration of deeper elements of the epidermis by fluores-

cent material was an exceptional finding. Usually no abnormal (*i.e.*, methylcholanthrene) fluorescence remained after the keratin crust flaked off at 4 to 8 days. The fluorescence in the subcutaneous fat cells became more diffuse within 1 to 2 days and gradually disappeared, all traces usually being gone by 6 to 8 days.

The paper is illustrated by fluorescence photomicrographs.—Authors' abstract.

A Cancerogenic Extract from Human Bile and Gall Bladders. Steiner, P. E. [*Univ. of Chicago, Chicago, Ill.*] Proc. Soc. Exper. Biol. & Med., **51**:352-353. 1942.

Human gall bladder bile and gall bladders were obtained from adults about 40% of whom died with malignant tumors. The dried residue (2,400 gm.) was saponified with alcoholic potassium hydroxide by refluxing on a steam bath for 24 hours. After repeated extractions with ethylene dichloride, the extracts were combined and dried over anhydrous sodium sulfate. The extract was then filtered and the ethylene dichloride distilled in partial vacuum. The residue was resaponified with alcoholic potassium hydroxide for 4 hours, and then extracted, dehydrated, filtered, and distilled, yielding a nonsaponifiable residue of 38.5 gm. Fifty-three mice, 107 to 127 days old, were injected subcutaneously, each receiving 250 mgm. of the extract in 0.75 cc. of sesame oil. The injections were repeated at 6 weeks. Mice of this stock have no spontaneous sarcomas other than lymphosarcomas.

Five sarcomas appeared at the site of injection in mice dying in the 14th, 20th, 23rd (2 mice), and 24th months after the first injection. The tumors were spindle, or spindle and mixed cell sarcomas, and resembled, both in morphology and growth, the subcutaneous sarcomas induced by carcinogenic chemicals in other experiments. —M. B.

The Incidence of a Carcinogenic Factor in the Livers of Cancer, Noncancer, Cirrhotic, and Negro Patients. Steiner, P. E. [*Univ. of Chicago, Chicago, Ill.*] Cancer Research, **3**:385-395. 1943.

Individual extractions and tests for carcinogenicity performed on 67 human livers, in which 896 mice were used, gave the following results: The presence of a carcinogenic factor in extracts of human liver was again demonstrated. It was present in livers from both cancer and noncancer persons. The yield of induced sarcomas was 2.9% in all mice that survived for 6 months. It was 13.1% in those experiments in which sarcomas were induced. Fourteen extracts out of 67 tested (about 21%) had carcinogenic activity. The induction time for the first tumor was 6 months. Eight extracts from cancer patients of 37 tested were carcinogenic (21.6%), while 6 of 30 noncancer extracts were carcinogenic (20.0%). Carcinogenic activity in the extracts was not related to any special site of tumor, or to any type, except that 4 of the 14 active extracts were from persons whose major disease was related to the endocrine glands concerned with steroid hormones. The incidence of the carcinogenic factor was about equal in all age groups, in the two sexes, in whites and Negroes, and in cirrhotic and noncirrhotic livers. The livers that showed the carcinogenic factor contained less extract on the average than did those that were inactive. Other experimental factors, including the number of injections, the stock of mice and possibly the type of solvent, did not appear to influence the results. The sarcomas were spindle and mixed cell, with no unusual features. The number of tumors at distant sites was not increased over those in several control groups. The carcinogenic activity in extracts of human liver has not been shown to be due to a preformed carcinogen, although this possibility is not excluded. It might be of importance even if it were a chemical conversion product.—Author's summary.

Photometric Histochemical Determination of Thymonucleic Acid in Experimental Epidermal Carcinogenesis. Stowell, R. E. [*Barnard Free Skin and Cancer Hosp., St. Louis, Mo.*] J. Nat. Cancer Inst., **3**:111-121. 1942.

Sections of mouse skin were stained for thymonucleic acid by the Feulgen technic. Quantitative measurements of the thymonucleic acid were then made with a photoelectric microphotometer. Comparison was made of the thymonucleic acid in the skin (1) of normal mice, (2) of mice that had been painted with pure benzene, and (3) of mice that had been painted with a 0.6% solution of methylcholanthrene in benzene. There was considerable variation between different parts of the same specimens, but, on the average, normal skin showed a higher thymonucleic acid content per unit volume than did skin that had been painted with benzene or with methylcholanthrene in benzene. The thymonucleic acid content per unit volume of papillomas and carcinomas was variable, being sometimes greater and sometimes less than that of normal skin. The mean amount of thymonucleic acid per cell was similar for most tissues.—H. Q. W.

Failure of Yellow O.B. to Produce Neoplasms. Sugiura, K. [*Memorial Hosp., New York, N. Y.*] Proc. Soc. Exper. Biol. & Med., **50**:214-215. 1942.

Yellow O.B. (1-*o*-tolylazo-2-naphthylamine) is an oil-soluble coal tar dye used to color foodstuffs. Twenty cubic centimeters of a 6% solution of this substance in olive oil was mixed with 1,000 gm. of ground, unpolished Texas rice. The basal diet was supplemented with about 1 gm. of fresh carrot per rat daily. Unlimited water was permitted. Thirty-one young adult rats (weight about 125 gm.) of the Sherman stock were used.

The daily ingestion of 7 to 12 mgm. of the dye by each animal failed to produce liver cirrhosis or tumors during 78 to 259 days of feeding.—M. B.

Effects of Carcinogenic Agents on Paramecium caudatum. Tittler, I. A., and Kobrin, M. [*Brooklyn Coll., Brooklyn, N. Y.*] Proc. Soc. Exper. Biol. & Med., **50**:95-96. 1942.

The carcinogens employed were methylcholanthrene (0.1 to 1%), scarlet red (0.1 to 1%), 3,4-benzpyrene (0.1 to 1%), and more dilute concentrations up to 1 part carcinogen in 100,000 parts of medium. The carcinogens were suspended in hay infusion cultures of *Paramecium caudatum* in Syracuse dishes. Subcultures of experimental and control animals were made at regular intervals; organisms were also fixed and stained for study.

In all the experimental cultures abnormalities such as swellings, vacuolizations of the cytoplasm, and blisterings of the pellicle occurred. Monster formations were not seen. The abnormal organisms lived for only 5 to 8 days, even after transference to a normal medium. There was no indication that either the macronucleus or micronucleus was involved.

3,4-Benzpyrene was the most potent with regard to the number of abnormalities produced and the rapidity of their production. Methylcholanthrene was next in potency, and scarlet red least.—M. B.

Endocrine Factors Influencing Tumor Development; Administration of Gonadotropins at the Early Cancer Age to Marsh-Buffalo Mice. Bischoff, F., Rupp, J. J., and Clarke, G. J. [*Santa Barbara Cottage Hosp. Research Inst., Santa Barbara, Calif.*] ENDOCRINOLOGY, **31**:329-333. 1942.

In virgin female mice of the Marsh-Buffalo strain, the administration of 100 Cole-Saunders units of pregnant mare serum hormone per mouse, initiated at the age of 7 months (the early cancer age) and repeated through a 3 month period, produced definite ovarian stimulation with pronounced alveolar development of the mammary gland but failed to alter appreciably the course of appearance of mammary gland tumors. Administration of 12 mgm. per mouse of sheep pituitary gonadotropic hormone at the same age and over the same period produced definite ovarian stimulation with pronounced alveolar development of the mammary gland after the first series of injections, followed by a 2 month period of induced atrophy of the ovaries and mammary glands. The course of the development of tumors of the mammary gland was not appreciably altered.—C. A. P.

A Spectrochemical Study of Estrogen-Induced Mammary Cancer in Mice. I. Chemical Preparation of Tissue and Analysis by Spectrophotometry. Strait, L. A., McCawley, E. L., and Perry, I. H. [*Univ. of California Med. Sch., San Francisco, Calif.*] CANCER RESEARCH, **3**:370-377. 1943.

In order to focus the study of the mechanisms by which estrogen induces mammary cancer a spectrochemical method of microanalysis in tissues of a synthetic estrogen, triphenylethylene (α-phenylstilbene), has been developed. Its absorption spectrum has been measured. The preliminary chemical preparation of tissues to be analyzed spectrographically is explained. A method of quantitative spectrophotometry with a single-beam, Baly tube, and densitometer is described, illustrated, and compared with the more customary split-beam methods. The application of this method of analysis to measurement in various tissues, tumor and mamma in particular, is illustrated. *In vitro* 0.05 mgm. per gm. of tumor tissue is measurable and 0.025 mgm. per gm. would be detectable. In mammary tissue 0.4 mgm. per gm. sample was measurable. The recovery of one-tenth of the estrogenic dose (0.5 mgm.) of triphenylethylene from 0.3 gm. of mammary tissue *in vitro* was 97.5% with a probable error of ±2.7%.—Authors' abstract.

A Spectrochemical Study of Estrogen-Induced Mammary Cancer in Mice. II. Distribution of Triphenylethylene in the Mamma and in Mammary Cancer Induced by This Estrogen. Perry, I. H., Strait, L. A., and McCawley, E. L. [*Univ. of California Med. Sch., San Francisco, Calif.*] CANCER RESEARCH, **3**:378-384. 1943.

Because of the obscurity regarding the role of estrogen in the genesis of mammary cancer, a spectrochemical study has been made to determine whether carcinogenic doses of a synthetic estrogen, triphenylethylene (α-phenylstilbene), administered to mice, reach the mammary tissue or the induced cancer, and whether previously untreated and repeatedly treated mammae differ in estrogen consumption.

By the use of the absorption spectrum, the estrogen triphenylethylene can be detected in the mammary tissue of mice in amounts that are of the order of one-tenth of the estrogenic dose and 1/2300 of the minimal administered dosage producing mammary cancer. The induced cancers and accompanying noncancerous but repeatedly treated "precancerous" mammae have been analyzed spectrochemically and four significant observations made: Triphenylethylene was not found in the tumor itself regardless of its state of development or the time when it was excised after administration of the last dose of triphenylethylene. Although triphenylethylene was not evident in the cancer, it was present in appreciable amounts in the remaining hyperplastic precancerous mammary tissue of the same animal if this was excised within 3 days following the last administered dose. It was not present in that removed after 7 days. The estrogen was not observed in previously untreated mammae 2 to 3 days after a single estrogenic dose (while the mice were still in estrus). Although extracts of the tumors studied exhibited without exception a characterless absorption spectrum, the hyperplastic precancerous mammary tissue from these animals exhibited characteristic new absorption bands in 5 of 18 cases. Further investigation is necessary to determine the origin and significance of these bands.

These observations suggest that: The mammary cancer induced by triphenylethylene does not absorb and retain triphenylethylene as does the hyperplastic precancerous gland. The estrogen seems rather to play a direct role in carcinogenesis preceding the development of the cancer. The apparent decreased ability of repeatedly treated mammary tissue to eliminate estrogen as compared with that subjected to a single dose of the estrogen may be an objective criterion of the precancerous state.—Authors' abstract.

Biocatalysts in Cancer Tissue. III. Succinic Dehydrogenase and Cytochrome Oxidase. Schneider, W. C., and Potter, V. R. [*Univ. of Wisconsin Med. Sch., Madison, Wis.*] CANCER RESEARCH, **3**:353-357. 1943.

The succinic dehydrogenase and the cytochrome oxidase activities have been determined for seven normal rat tissues and for 10 kinds of experimental tumors. The Q_{O_2} values for these enzymes in tumor tissues were considerably lower than those for normal tissues as a whole; the Q_{O_2} values in tumors were quite constant regardless of etiology, whereas in normal tissues they varied more widely. Liver tumors had only about one-fourth of the succinic dehydrogenase activity and about

one-third of the cytochrome oxidase activity of normal liver. Homogenization of the tissue in water increased the cytochrome oxidase activity above that observed in tissues homogenized in isotonic phosphate buffer; this procedure had no appreciable effect upon the succinoxidase activity. The available evidence suggests that tumor tissues do have a deficient type of oxidative metabolism but that the succinoxidase system is not the weakest link in the oxidative cycle.—Authors' abstract.

Biocatalysts in Cancer Tissue. IV. An Enzyme-Virus Theory Regarding Carcinogenesis. Potter, V. R. [*Univ. of Wisconsin Med. Sch., Madison, Wis.*] CANCER RESEARCH, **3**:358-361. 1943.

A theory of carcinogenesis is proposed, suggesting that cancer may be the result of a competition between an enzyme X and a "cancer virus," the latter being derived from the former by action of carcinogenic agents. The identification of enzyme X is considered to be of prime importance in the solution of the cancer problem. Some of the hypothetical properties of enzyme X are compared with the observed properties of succinic dehydrogenase.—Author's abstract.

Uniformities in the Content of B Vitamins in Malignant Neoplasms. Taylor, A., Pollack, M. A., and Williams, R. J. [*Univ. of Texas, Austin, Tex.*] SCIENCE, **96**: 322-323. 1942.

If in a number of samples of tissue the vitamin content is found to have a standard deviation of 20% of the mean level, there would be an average degree of uniformity of this vitamin of 80%. The average of all the "vitamin uniformities" so expressed in a series of tissues calculated individually for 8 B vitamins is called the "total B vitamin uniformity."

Normal tissues of the same type but taken from separate animals have a relatively high "total B vitamin uniformity" (70% or more), while normal tissues that differ in structure and function have a relatively low degree (less than 30%) of such uniformity.

Malignant tumors differing in tissue of origin, manner of induction, and host species tended to a high "total B vitamin uniformity" when compared with one another. Since a high degree of such uniformity was found associated only with homogeneous and never with heterogeneous groups of tissues, it is concluded that malignant neoplasms of various types and from various animals tend to have similar cellular metabolism, and constitute a common tissue type.—M. B.

A Rapid Test for Tumor Growth Inhibitors. Laszlo, D., and Leuchtenberger, C. [*Mt. Sinai Hosp., New York, N. Y.*] CANCER RESEARCH, **3**:401-410. 1943.

The determination of the inhibition of tumor growth is established by comparing tumor sizes and tumor weights of treated groups with untreated controls at the end of an experimental period of 48 hours. The growth rate of sarcoma 180 is used as the indicator. Seven to 10 days after transplantation batches of at least 7 mice each with comparable tumor size are selected. One group is injected intravenously twice daily for 2 consecutive days with the material to be tested, another with a standard of reference, and a third with saline for control. Forty-eight hours later, the tumor sizes and tumor weights are determined. The technic and the conditions for the standardization are described in detail and the material is analyzed statistically. The practical application of this rapid test for the detection of tumor growth inhibitors in various sources, such as brewer's yeasts, grains, and pure chemicals, and its use for tracing inhibitory activity of chemical fractions are demonstrated.—Authors' abstract.

The Effect of Rate of Freezing on the Survival of Fourteen Transplantable Tumors of Mice. Snell, G. D., and Cloudman, A. M. [*Jackson Memorial Lab., Bar Harbor, Maine*] CANCER RESEARCH, **3**:396-400. 1943.

In preliminary tests it was found that several transplantable tumors would survive immersion in isopentane or freon 11 at room temperature for 30 minutes without reduction in the percentage of takes. Comparisons were then made of the effects on survival, of rapid and slow freezing. Direct immersion of small pieces of tumor tissue in isopentane chilled to about −75° C. with dry ice was used to induce rapid freezing. For slow freezing, small pieces of the same tumors were placed in isopentane in a vial at room temperature and the vial and its contents then cooled to −75° C. by placing on dry ice in a thermos jug. Thawing was accomplished by immersion of the tissues in Locke's solution at 33–38° C. Of 14 tumors thus tested, 4 failed to grow following either treatment, 8 grew better following slow freezing, 2 following fast freezing.—Authors' abstract.

Diplochromosomes in a Goldfish Tumor. Biesele, J. J. [*Univ. of Texas, Austin, Tex.*] CANCER RESEARCH, **3**:411-412. 1943.

The chromosomes in an ovarian tumor of a goldfish had an average volume double that of the chromosomes in a healing skin wound and in nongerminal cells of a normal ovary. The maximum number of nucleoli was 4 in the normal nuclei and 8 in the cancerous. Hence the chromosomes of the goldfish tumor must be regarded as diplochromosomes. This investigation is an extension of work on mouse cancers, in which analogous observations have been made.—Author's abstract.

Clinical and Pathological Reports

Therapy—General

Control of Pain in Cases of Cancer. Greenhill, J. P. [*Cook County Hosp., Chicago, Ill.*] M. Clin. North America, **25**:117-128. 1941.

Five methods are discussed for relieving or preventing pain in cases of cancer. These are: (1) the use of opiates, (2) the administration of cobra venom, (3) intraspinal injection of alcohol, (4) sympathectomy, and (5) cordotomy.

Other methods that are not considered as acceptable as those mentioned above include: (1) refrigeration, (2) hibernation, (3) intravenous injection of 33% ethyl alcohol solution, and (4) intravenous, intramuscular, and oral administration of calcium gluconate.—J. L. M.

Pain in Cancer of the Face, Jaws, and Neck. An End-Result Study of the Relief Afforded by Neurosurgical Methods. Munro, D. [*Harvard Med. Sch. and Boston Univ. Sch. of Med., Boston, Mass.*] New England J. Med., **224**:1049-1053. 1941.

A discussion of 30 cases.—G. H. H.

Radiation—Diagnosis and Therapy

Observations on the Results of Combined Fever and X-Ray Therapy in the Treatment of Malignancy. Shoulders, H. S., Turner, E. L., and Scott, L. D. [*Meharry Med. College, Nashville, Tenn.*] South. M. J., **35**: 966-970. 1942.

The combined technics seem to produce results definitely superior to those obtainable by x-rays alone.—H. G. W.

Chorionepithelioma in the Male and Female as Observed Roentgenologically. Arendt, J. [*Mt. Sinai Hosp., Chicago, Ill.*] Am. J. Roentgenol., **47**:591-595. 1942.

Two cases of chorionepithelioma of extragenital origin in the male are presented with one autopsy. Both were diagnosed roentgenologically from the appearance of the lung metastases. The metastases are well defined, nodular, and concentric, but differ from sarcoma metastases in that the margins blend with the surrounding tissue in a softly blurred outline due to hemorrhage. A description of the various forms in which chorionepithelioma might occur in the roentgenogram is given.—C. E. D.

Five Year Control of Bladder Cancers by Radon Implants. Barringer, B. S. [*Memorial Hosp., New York, N. Y.*] J.A.M.A., **120**:909-911. 1942.

Bladder cancers, both papillary and infiltrating, are well adapted for attack by radon implants. In 257 cases seen up to and including 1937, there were 112 papillary and 145 infiltrating cancers. Excluding 15 cases, in most of which there were metastases and palliative operation was performed, 56.1% of patients with papillary cancer and 28.9% with infiltrating cancer have remained well for 5 years. For both groups combined there is a 5 year cure rate of 40.4%. While tumors of the bladder vault and extensive papillomatosis are better dealt with by surgery alone, in all other bladder cancers radon implantation is simpler to effect, has a lower operative mortality, and gives more assurance of 5 year cure.—H. G. W.

Radium in Medicine: Introduction and General Considerations. Fricke, R. E. [*Mayo Clinic, Rochester, Minn.*] M. Clin. North America, **25**:873-884. 1941.

A review on the use of radium in the treatment of cancer including the following subjects: discovery of the x-ray and early investigation of uranium, discovery and properties of radium, source and supply of radium, radium therapy, and radiologic associations and publications.—J. L. M.

Radium Therapy for Carcinoma of the Female Genitalia. Fricke, R. E. [*Mayo Clinic, Rochester, Minn.*] M. Clin. North America, **25**:905-914. 1941.

This review is concerned with the treatment of carcinomas of the ovary, fallopian tube, uterine cervix, uterine fundus, vagina, and vulva. In the fortunate instances in which diagnosis can be established early and in which the general condition of the patient is favorable, radical surgical operation followed by irradiation is the treatment of choice.—J. L. M.

Treatment of Nonmalignant Conditions with Radium. Fricke, R. E. [*Mayo Clinic, Rochester, Minn.*] M. Clin. North America, **25**:945-956. 1941.

In general, treatment with radium of all benign lesions, whether neoplastic or inflammatory, necessitates certain precautions. Most of these conditions are not fatal if not treated. By overtreatment, underfiltration of radium, or lack of protection to adjoining tissues, a benign condition may be changed into a malignant one. Unskilled treatment may cause serious damage to the skin and underlying tissues, necessitating surgical repair. It should be mentioned that for benign conditions good results can be achieved with only a percentage of the dose used in the treatment of carcinoma. The treatment is never a full erythema dose.—J. L. M.

Dangers of Radiation without Biopsy of Brain Tumors in Children. Report of a Case. Ingraham, F. D., and Campbell, J. B. [*Harvard Med. Sch., Boston, Mass.*] New England J. Med., **224**:925-927. 1941.

A case report concerning a patient treated for 2 years with radiation for a cerebellar tumor thought to be a medulloblastoma. During the period of treatment permanent blindness ensued. At operation a fibrillary astrocytoma was removed. The authors emphasize the uncertainty involved in classification of brain tumors without benefit of biopsy.—G. H. H.

Carcinoma of the Cervix. Clinical Evaluation of Radium Dosage and Supplementary Roentgen Irradiation Based on a Study of 915 Cases. Jones, H. W. [*Kelly Clinic, Baltimore, Md.*] South. M. J., **35**:959-965. 1942.

A clinical evaluation of radium dosage and supplementary roentgen irradiation, based on a study of 915 cases.—H. G. W.

Recent Advances in Radiation Therapy. Kaplan, I. I. [*New York Univ. Med. Coll., New York, N. Y.*] M. Clin. North America, **25**:803-814. 1941.

The history of radiation therapy is traced from the time of the discovery of x-rays by Roentgen in 1896. The following items which led to the standardization of radiation

therapy are briefly discussed: rate of administration, irradiation by several converging beams (crossfire method), divided dose method, saturation method, radium and radon therapy, interstitial irradiation with radon seeds and tubules, radium pack therapy, and preoperative irradiation. The use of irradiation in the treatment of various neoplasms is briefly mentioned. The application of nuclear physics (artificial radioactive elements and neutrons) is still in its early stages, and clinical results are too uncertain to make evaluation of this therapeusis possible.—J. L. M.

The Roentgen Ray Treatment of Malignant Tumors. Leddy, E. T. [*Mayo Clinic, Rochester, Minn.*] M. Clin. North America, **25**:973-1009. 1941.

The paper reviews the use that may be made of roentgen rays by the general practitioner. Several problems of technic are considered, and the roentgen ray treatment of tumors is discussed. The radiosensitivity of tumors parallels that of their normal cell prototypes. This permits subdivision of all tumors into 4 classes. (1) Those which theoretically and under ideal conditions can be cured by roentgen rays because they are radiosensitive (lymphoblastoma, leukemia, carcinoma of the cervix, intraoral carcinoma, carcinoma of the larynx). (2) Those in the treatment of which roentgen rays should be combined with other methods (tumors of the nervous system; malignant tumors of the eye, salivary glands, lymph nodes of the neck, and thyroid gland; carcinoma of the breast, bronchi, bladder, rectum; and malignant tumors of the kidney, testis, ovary, and bone). (3) Those in the treatment of which roentgen rays are of questionable or no value (carcinoma of the gastrointestinal tract and fundus of the uterus, fibroid tumors, periosteal sarcoma, malignant melanoma, carcinoma of the prostate gland). (4) Those in which treatment by roentgen rays is still experimental.—J. L. M.

The Lesions Produced in the Gastro-Intestinal Tract by Irradiation. General Review with an Illustrative Case Report. Mulligan, R. M. [*Univ. of Colorado, Sch. of Med. and Hosps., Denver, Colo.*] Am. J. Path., **18**:515-527. 1942.

The clinical and experimental studies on radiation lesions of the gastrointestinal tract are reviewed, and a case is described.—J. G. K.

Diaphragmatic Abnormalities Secondary to Tumors. A Roentgenologic Study. Turner, J. W. [*Westfield State Sanatorium, Massachusetts Dept. of Public Health, Mass.*] New England J. Med., **224**:936-940. 1941.

Three instances of diaphragmatic abnormalities, determined by x-ray, are reported: left subphrenic abscess due to a perforated carcinoma of the stomach, paralysis of the right phrenic nerve due to a carcinoma of the bronchus, and left diaphragmatic hernia apparently caused by increased intra-abdominal pressure due to a large cystic hemangioendothelioma.—G. H. H.

Pathology and Pathologic Diagnosis of Radiation Lesions in the Gastro-Intestinal Tract. Warren, S., and Friedman, N. B. [*Harvard Med. Sch., Boston, Mass.*] Am. J. Path., **18**:499-513. 1942.

Thirty-eight cases were studied in which pronounced radiation lesions in the gastrointestinal tract followed radiotherapy. The lesions consisted of ulceration, sclerosis, and combinations of the two. Necrosis was a part of most reactions, and in extreme instances the reaction approached massive gangrene of a loop of intestine. Histopathologically, the primary points to be looked for in radiation lesions are hyalinization of the connective tissue, abnormal fibroblasts, telangiectasia, and hyaline degeneration of vessel walls. These are described in detail and illustrated.—J. G. K.

Nervous System

Intraspinal Meningiomas. A Clinical and Pathologic Study. Brown, M. H. [*Mayo Foundation, Rochester, Minn.*] Arch. Neurol. & Psychiat., **47**:271-292. 1942.

In the investigation reported, 130 meningiomas of the spinal cord were examined both clinically and pathologically. In this group, the following 8 variants have been distinguished: meningothelial, fibroblastic, psammomatous, osteoblastic, lipomatous, chondromatous, melanomatous, and, finally, malignant. It is concluded that meningioma is the product of an admixture of 2, and at times of 3, different elements; *e.g.*, the specialized arachnoid type cell, its multipotential stroma, and dural components. The neoplastic cells *per se* arise from the superficial layer of the arachnoid, stimulate its stroma, which is likewise of neural crest origin, and incorporate mesenchymal elements into the tumor pattern only as a result of the usually rapid dural attachment for purposes of vascularity. Diffuse meningomatosis, the neoplastic transformation of embryonically arrested leptomeninges, has been delineated in a separate category and differentiated from gliomatosis, sarcomatosis, and melanomatosis of the meninges.—A. C.

Effects of Destruction of Hypothalamus by Tumor. Collins, V. P. [*New England Deaconess Hosp., Boston, Mass.*] Arch. Neurol. & Psychiat., **48**:774-788. 1942.

This is a report of the clinical and pathologic manifestations of a slowly growing tumor involving the floor and walls of the third ventricle in such a manner as to destroy all nuclei of the hypothalamus and to sever functionally the hypophysial stalk, while not disturbing adjacent regions or obstructing the flow of cerebrospinal fluid. Early symptoms may have been present in infancy; pronounced polydipsia and polyuria were first noted when the patient was 17. The various stages of the disease, which was terminated by the death of the patient at the age of 28, included diabetes insipidus; suppression of function of the pituitary (anterior lobe), the thyroid, the ovaries, and the adrenal glands; disturbance of thermal regulation; and disturbance of personality. The case is discussed, and it is concluded that the manifestations just mentioned can be attributed to the destruction of the hypothalamus by the tumor.—A. C.

Lindau-von Hippel Disease. A Report of Four Cases. Craig, W. McK., Wagener, H. P., and Kernohan, J. W. [*Mayo Clinic, Rochester, Minn.*] Arch. Neurol. & Psychiat., **46**:36-54. 1941.

A report of 4 cases of hemangiomas of the cerebellum associated with angioma of the retina. The family history in one of the cases, illustrated by a genealogic chart,

supports the view that the disease is of congenital origin.—A. C.

Multiple Meningioma. Removal of Ten Intracranial Tumors from a Patient. Echols, D. H. [*Tulane Univ. Sch. of Med., New Orleans, La.*] ARCH. NEUROL. & PSYCHIAT., **46**:440-443. 1941.

A case report.—A. C.

Removal of Tumor Arising Anterior to the Medulla. Ecker, A. [*Syracuse Univ. Coll. of Med., Syracuse, N. Y.*] ARCH. NEUROL. & PSYCHIAT., **46**:908-912. 1941.

The tumor, a meningioma arising from the anterior rim of the foramen magnum, had displaced the medulla and spinal cord backward. This case is believed to be the second reported in which removal of the tumor was apparently complete, with recovery of the patient.—A. C.

Mixed Tumors of the Spinal Canal. French, L. A., and Peyton, W. T. [*Univ. of Minnesota Hosps., Minneapolis, Minn.*] ARCH. NEUROL. & PSYCHIAT., **47**:737-751. 1942.

Depending on the number of germ layers present, mixed tumors may be classified into the 3 following types: teratomas, dermoids, and epidermoids. The paper reports on the diagnosis and treatment of 3 cases of mixed tumors of the spinal canal, including 2 teratomas and 1 epidermoid cyst.—A. C.

Metastatic Tumors of the Brain. Globus, J. H., and Meltzer, T. [*Mt. Sinai Hosp., New York, N. Y.*] ARCH. NEUROL. & PSYCHIAT., **48**:163-226. 1942.

The clinical and anatomic manifestations in 57 cases of metastatic tumors of the brain were analyzed. The series constitutes about 13.5% of the entire collection of brain tumors encountered at necropsy at the Mount Sinai Hospital. On the basis of the present study it may be said that the following features point strongly to the presence of an expanding brain lesion of metastatic nature: (1) acute onset of symptoms indicative of increase in intracranial tension, (2) rapid development of neurological signs but slow development of papilledema, (3) absence of positive serological reactions and febrile manifestations. The presence or absence of meningeal signs does not seem to bear any relation to the position of the tumor in the brain. Anatomic and microscopic study of the material suggests that the metastatic cells were brought to the brain by way of the blood stream.—A. C.

Otitic Thrombosis of the Cerebral Sinuses and Veins Simulating Multiple Brain Tumors. Keschner, M., and Davison, C. [*Montefiore Hosp., New York, N. Y.*] ARCH. NEUROL. & PSYCHIAT., **47**:428-437. 1942.

A report of a case in which thromboses and hemorrhages in the region drained by the right and the left middle and posterior cerebral veins simulated multiple tumors of the brain. A definite diagnosis could not be established until necropsy.—A. C.

Simmonds' Disease: Report of Two Cases Caused by Intracranial Tumors. Moss, R. E. [*Boston Univ. Sch. of Med., Boston, Mass.*] J. CLIN. ENDOCRINOL., **2**:395-402. 1942.

Detailed case reports and postmortem data are presented for an 18 year old boy and a 70 year old man who had intracranial tumors and evidence of Simmonds' disease. The younger patient had diabetes insipidus.—J. B. H.

Pain Arising from Lesions of the Nerves and Spinal Cord: Differential Diagnosis and Treatment. Oldberg, E. [*Univ. of Illinois, Coll. of Med., Chicago, Ill.*] M. CLIN. NORTH AMERICA, **25**:55-62. 1941.

Eight cases are presented to illustrate specific types of pain arising from lesions affecting nerves rather than the spinal cord. In these the pain arose from extramedullary intradural spinal tumor, tabetic radiculitis, postherpetic neuritis, peripheral avitaminotic neuritis, peripheral neurofibroma, neuritis resulting from focal infection, cervical rib, and protruded intervertrebal disc. Differential diagnosis and treatment are discussed.—J. L. M.

Epidermoid, Dermoid and Teratomatous Tumors of the Central Nervous System. Peyton, W. T., and Baker, A. B. [*Univ. of Minnesota, Minneapolis, Minn.*] ARCH. NEUROL. & PSYCHIAT., **47**:890-917. 1942.

Fourteen cases are reported.—A. C.

Head Pain: Differential Diagnosis and Treatment. Pollock, L. J. [*Northwestern Univ. Med. Sch., Chicago, Ill.*] M. CLIN. NORTH AMERICA, **25**:3-13. 1941.

Head pain from intracranial tumors is discussed. Five case histories are given to illustrate the fact that although headache may be the presenting symptom, it is not the sole symptom in intracranial tumors. Several conditions other than tumors are referred to.—J. L. M.

INTRATHORACIC TUMORS—LUNGS—PLEURA

Carcinoma of the Lung: Bronchoscopic Aspects. Betts, R. H. [*New England Deaconess Hosp., Boston, Mass.*] NEW ENGLAND J. MED., **225**:519-525. 1941.

A general discussion with the presentation of 62 patients with histologically verified pulmonary tumors. In 74% the diagnosis was established by bronchoscopic biopsy. —G. H. H.

Commercial Lead as a Possible Inciting Factor in Bronchiogenic Carcinoma. Report of Two Cases. Black, C. E. [*Michigan State Coll., East Lansing, Mich.*] ARCH. PATH., **35**:366-372. 1943.

Bronchiogenic carcinoma is reported in 2 lead workers, both of whom showed also chronic fibroid pneumonitis. Because commercial lead is the most radioactive of the common metals, it may be a possible factor in the production of lung cancer when lead dusts are inhaled.—H. G. W.

Extragenital Chorio-Epithelioma in the Male with Associated Gynecomastia. Bonn, H. K., and Evans, N. [*Los Angeles County Hosp., Los Angeles, Calif.*] AM. J. SURG., **58**:125-132. 1942.

This patient, a man of 34, presented what seems to be the sixth case on record in which proof of extragenital origin of chorionepithelioma was furnished by serial block sections of the testes. Possibly the primary focus was located at the hilus of the lung. Abnormal amounts of sex hormones were found in the urine and in the metastases in the lungs, and a true gynecomastia was present in both breasts.—H. G. W.

Thoracic Surgery. Churchill, E. D. [*Harvard Med. Sch., Boston, Mass.*] NEW ENGLAND J. MED., **225**:335-338. 1941.

A brief discussion of the pathogenesis and treatment of bronchial adenoma, blast injuries to the chest, carcinoma and congenital atresia of the esophagus, cardiospasm, and bronchiectasis.—G. H. H.

Book Reviews

THE ADRENAL CORTEX IN ADAPTATION TO ALTITUDE, CLIMATE, AND CANCER. By Edward S. Sundstroem and George Michaels [formerly Giragossintz]. Memoirs of the University of California. Vol. 12. University of California Press, Berkeley and Los Angeles. 1942. VIII + 410 pages; 125 illustrations. Price $4.00.

After two preliminary chapters on the method employed and the response of normal rats to low atmospheric pressure, the authors take up its effect on tumors of various sorts.

At 300 and 360 mm. definite damage was found in transplantable tumors of five different strains. Not only was growth slower and necrosis more extensive than under normal conditions, but complete regression was much more common. This occurred, also, among strains in which it is ordinarily rare and that resist successfully most other therapeutic measures. But with commendable caution the authors interpret these effects merely as manifestations of the tendency to self cure possessed in greater or less degree by all neoplasms in this group.

The few spontaneous growths used were chiefly mammary adenocarcinomas of the mouse. Exposed to pressures of 260 and 420 mm. they were not quite so easily affected, though the results are described as not wholly discouraging. Other experiments suggested that carcinogenesis in animals with a cancerous heredity might, perhaps, be a little delayed.

The development of tar cancer in mice (the synthetic carcinogenic hydrocarbons were not yet available) appeared to be somewhat slower under the treatment, and the tumors may have been a little less malignant, but on the whole the process was not greatly affected.

What slight damage was done to the tumor cell is ascribed to cooperating deficiencies in oxygen and the hormone of the adrenal cortex.

A wealth of charts illustrate the book, together with a few drawings, photographs, and photomicrographs, and an adequate bibliography is supplied.

The authors do not lack the faculty of self criticism and to the reviewer, as to them too, no doubt, the results, at least so far as tumors are concerned, seem to fall far short of what their enormous industry justly deserved.

William H. Woglom

ATLAS OF OVARIAN TUMORS. By Gemma Barzilai, M. D. Grune & Stratton, New York. 1943. 261 pages; 58 plates. Price $10.00.

This book represents a new form for the presentation of material on gynecologic pathology. The chief emphasis is placed on histologic description and on photomicrographs and colored plates. These have been exquisitely reproduced and offer what is perhaps the finest source available of pictures of ovarian tumor pathology. By the evident intent of the author references to the literature and clinical aspects have been reduced to a minimum. Here then is a work devoted to the description in minutest detail of the varying structure of ovarian neoplasms.

According to the author the photomicrographs are based upon material used to illustrate her lectures in Padua, and represent slides from her own collection as well as from material placed at her disposal in Vienna, Istanbul, and Milan.

The classification follows what is gradually coming to be an accepted pattern, with some changes in arrangement and emphasis and some new terms. Granulosa cell and theca cell tumors, perhaps logically as tumors of the specific ovarian structures, have been elevated to the head of the list. There follow the arrhenoblastoma and a tumor that the author cautiously terms a "virilizing lipoid tumor." The concept of derivation from specific embryonal structures or from a one-sided development of a teratoma is given considerable stress and is applied to the majority of the ovarian tumors. One term new to most American gynecologists is the "endosalpingioma," the tumor usually designated a serous, papillary cystadenoma. The morphologic similarity of the epithelium of this growth to that of the tube is made impressive by the author's detailed consideration of its histology. She suggests also that a less differentiated epithelial tumor of the ovary, which she calls the "seroanaplastic carcinoma," may be the malignant form of endosalpingioma.

The reviewer remains unconvinced that the morphologic similarity of this ovarian tumor to the tubal epithelium justifies the term endosalpingioma and somewhat doubts the wisdom of attempting to change the nomenclature now in use. This reservation, however, does not detract from Dr. Barzilai's contribution in drawing attention to the suggestive cellular structure of these neoplasms.

The atlas should serve as at least a partial answer to the need for a dependable work with illustrations of all types of ovarian tumors with which the pathologist may compare the daily material of his laboratory. The painstaking study that has gone into the text and the beauty of the illustrations will make this a desirable volume for the library of the gynecologist also.

Howard C. Taylor, Jr.

BIOCHEMISTRY AND MORPHOGENESIS. By Joseph Needham. Cambridge University Press, London. 1942. XVI + 787 pages; 328 illustrations. Price £2.12.6d.

Apart from its vast general significance, this book should prove of the greatest value to students of cancer on account of the author's characteristic treatment of various subjects relating to oncology. While it will amply repay reading and rereading *in toto*—as indeed it must be studied if the whole plan is to be appreciated—it contains a contents list, with a decimal classification, that is sufficiently detailed to guide the specialist in more particular topics.

Describing the origins of the notion of morphogenetic stimuli, Needham shows how, as early as 1858, Virchow developed this conception in relation to the production of specific types of tumor in man, and how the subject was further advanced by Billroth (1890), who attributed such effects to chemical substances elaborated by the stimulating organism, whether insect, worm, or bacterium. These matters are more fully considered in what Needham modestly calls a digression on the interesting and somewhat neglected subject of gall formation in plants.

Among other morphogenetic stimuli, the primary organizer of amphibian development has special relevance to the tumor problem, although Needham recognizes that this is not apparent at first sight (since evocator substances are concerned with specific differentiation and not with

general growth), and that much must depend on the extent to which the individuation field persists into the adult condition. If the capacity for regeneration is a suitable measure of such persistence, the mammalian individuation field has been wholly lost during development. (Concerning neoplasms in reptiles, anurans, and urodeles, it may be noted that a convenient summary is provided in Table 21, page 241.)

These qualifications aside, the chemical nature of the evocator must clearly be a matter of special interest. Needham admits the tendency in certain quarters, and quotes Spemann in this regard, to dismiss the whole induction effect as unspecific, an attitude that he deplores as a counsel of despair. But is not this tendency largely justified, inasmuch as neural tube inductions have been obtained following implantation of agents as diverse as 1,9-dimethylphenanthrene, methylcholanthrene, "styryl blue," 3,4-benzpyrene, anthracene, sitosterol, pregnandiol, and squalene, apart from fatty acids and nucleoproteins? Of course the author makes it perfectly clear that neural inductions can be brought about by a wide variety of chemical fractions and pure or relatively pure substances, and that the question which of these, if any, is identical with the primary evocator occurring in the dorsal lip of the blastopore, remains unanswered: "The question is rendered particularly difficult owing to the presence of the natural substance in masked condition in the very tissue on which alone the activity of a chemical substance can be tested." But one may perhaps be allowed to differ rather strenuously from Needham when he asserts that studies of dosage "apart from the direct evidence from solubility, etc.," indicate that the natural evocator is a steroid substance. The evidence he puts forward is that the only substance so far shown to act in concentrations "of the vitamin or hormone order" is a polycyclic hydrocarbon, actually an endosuccinate of 1,2,5,6-dibenzanthracene. This evidence is surely insufficient to warrant any such conclusion, even if we admit, as is suggested, that many of the other types of substance that have given positive results have probably done so by unmasking the natural evocator.

In a section on organizer excess and anomalous competence, Needham passes to the problem of teratomas. While appreciating the vastness of the literature, he regards a good deal of it as marked by unscientific speculation, inaccurate description, and errors of logic. Good individual cases, as, for example, some described by Willis and by Barnard, are selected to show the parallelism between teratomas and the chaotic distribution of tissues that is seen when the individuation field in a young embryo is thrown out of gear. While normal induction of the primary axis in an embryo involves both evocation (the stimulus of a chemical substance) and individuation (the regional differentiation of the axis so induced), a dead piece of organization center probably carries the evocator but not the individuation field. Hence it might be expected that the implantation of dead organizer should elicit the appearance of chaotically arranged structures, "always provided that the individuation field of the host was not sufficiently strong to control and order the newly appearing differentiations." It is not entirely clear to the reviewer to what extent this explanation is a true advance, or to what extent merely a restatement on the basis of analogy, however sound. One must nevertheless freely acknowledge the provocative value of the concept itself; namely, that the phenomena of teratoma formation are due to a failure of the individuation field, at some point early in development, to control the action of evocating substances. The subject is further discussed not only in its relation to spontaneous teratomas but also to the Michalowsky-Bagg teratomas induced by injection of zinc chloride into the testes of the fowl. It is interesting that Needham gives credit for the first application of organizer phenomena in teratoma research to Budde (*Beitr. z. path. Anat. u. allg. Path.*, **75**:357. 1926), who attributed these tumors to what he called an *abgesprengter Organisator,* in a paper written very shortly after that of Spemann and Mangold (*Arch. f. Entwicklngsmechn. d. Organ.*, **100**:599. 1924).

Proceeding to homoiogenetic induction (the power acquired by part of an embryo, at the same time as it is determined for a certain differentiation, to induce another of the same kind) the author elaborates what he believes is a formal analogy between this process and the propagation of these types of cancer transmissible by cell-free extracts. But here the analogy is surely much less happy. In particular, there is no positive evidence at present available to support his hypothesis that the Rous agent is a specific hydrocarbon-protein complex, and a great deal that is hardly consistent with such a view. Needham closes his account of organizers and cancer with a quotation from J. B. S. Haldane: "Until it is shown that differentiation is due to gene mutations, it seems reasonable to regard carcinogenesis as anomalous differentiation rather than mutation." This sentence, he believes, provides the justification for the discussion of cancer phenomena in a book on chemical embryology.

Most of the topics remaining, apart from a short account of hereditary tumors in *Drosophila* and various fishes, have a less specific or direct bearing on cancer but are nevertheless of the most profound significance. Thus an excellent account is given (Fig. 205) of the possible relationships, natural and experimental, between the nucleus and cytoplasm in development. This raises among other problems that of parthenogenetic merogony, in which phenomenon E. B. Harvey has demonstrated the possibility of segmentation and even blastula formation, in the complete absence of either paternal or maternal chromatin, in echinoderm eggs. Reference is also made to the question whether cleavage rate in echinoderms is a function of the cytoplasm or the nucleus, in an account of A. R. Moore's experiments with the cross *Dendraster* ♀ × *Strongylocentrotus* ♂, and with the fertilization, either by *Dendraster* or *Strongylocentrotus* sperm, of nucleate and nonnucleate fragments obtained by microdissection; in every case the cleavage rate was that characteristic of the cytoplasm.

The last part of the book, which deals with morphogenetic mechanisms, contains a good fundamental description of the dissociability of, or incompatibility between, the processes of growth and differentiation, referring among other authors to von Bertalanffy, who postulated a causal relationship between rising differentiation and falling specific growth rate, and to both Peter and Lauche,

who were led to conclude that "a cell which is working does not divide, and a cell in mitosis is not working."

A later section deals with the degree to which growth and differentiation are reversible, and whether in reversal they show the same phenomenon of dissociation. Needham indicates that there is no true dedifferentiation in the regression of planarians, as studied by F. R. Lillie in 1900 in *Planaria dorotocephala;* starvation of this organism produces a reduction in size to less than that at hatching, but the only morphological changes that occur are slight alterations of the proportions of the parts. The association of dedifferentiation with "degrowth" has, however, been long recognized among ascidians; for example, in the work of Julian Huxley on regression in *Clavellina.* Even here, however, the regressive changes were not looked on by Huxley as reversions to stages passed through in embryogenesis, and "no ascidian tadpole makes its appearance when an ascidian dedifferentiates." The regression is far from being a true reversibility, and is rather an assumption of the cuboidal or spherical shapes, *i.e.,* a condition requiring the least amount of energy for maintenance.

A parallel section deals with the dissociability of growth and metabolism (fermentation and respiration). Starting with the early observations of Warburg on the inhibition of cleavage (but not of respiration) of sea urchin eggs by hypertonic sea water and phenylurethane, it gives an account of many examples illustrating the disengagement of these processes in different types of developing embryonic cells, and unicellular organisms, under the influence of such agents as quinine, iodoacetate, heightened CO_2 tension, and irradiation with x-rays. For tumor tissue, the work of Crabtree with β- and γ- radiation is mentioned, but not that of Boyland (*Biochem. J.,* **33**:618. 1939), on the dissociation of growth and metabolism in spontaneous mammary tumors of the mouse as shown through the inhibition of growth by chemical means.

At the conclusion of a section on heterauxesis, Needham introduces the "sense of duration" and the general notion of "physiological time" as a factor in embryonic events, drawing attention to the contributions of Lambert and Teissier, among others, who proposed as a fundamental law of biology that homologies exist between animals in time as well as in space. "Mouse time must bear the same, or a similar, relation to elephant time as mouse spatial magnitudes to elephant spatial magnitudes. Indeed, unless the time factor is brought into account, we may understand morphological similarity, but we can never hope to understand physiological, still less embryological, similarity." The ratio borne by the latent time of carcinogenesis to specific life span is probably a special case, to which such considerations can be usefully applied.

Finally, under the section on protein metabolism there is included a description of the growth-promoting factor in its relation to cultivation *in vitro,* the healing of wounds, recovery from injury, and the growth of tumors.

The book is provided with a glossary of special terms. Only few pertain directly to cancer, and although the list makes no legislative claims at least one definition, that of metastasis as the "spontaneous dispersion of a tumor from its original site in the body to other sites," has an archaic ring and should be improved. One is pleased to see that a list of terms the use of which is not recommended, and which in the author's words deserve their *requiescat,* includes "blastogen" and "blastomogen" (for carcinogen).

A bibliography containing several thousand references is supplied, together with animal, plant, gene, and general indexes. The last is not a sufficiently good guide to individual compounds, and a separate chemical index might have been provided with advantage.

To conclude, it is obvious that in spite of limitations in regard to particularities, and these are perhaps not altogether avoidable, the book is one of the first importance for general biology and all its branches. This is not less so because of the breadth of vision that it exemplifies and inculcates, which is so desirable and indeed necessary in the proper study, among the other subjects with which the work deals, of the cancer problem in all its varied aspects.

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CANCER OF THE UTERUS. By E. Hurdon. Oxford University Press, London. 1942. XII + 188 pages.

A few months before her death in 1941 Dr. Elizabeth Hurdon, late Medical Director of the Marie Curie Hospital, London, left the manuscript of this book in the hands of Dr. L. Martindale and Professor S. Russ, who have arranged its publication and contribute a preface. A short introductory chapter deals with those general aspects of the cancer problem (incidence, nature and causation, hereditary and other predisposing factors) that have clinical implications for the specific topic. Cancer of the uterus (of cervix and corpus) is then considered in its clinical pathology, pathological histology with special reference to tumor grading, and clinical history and diagnosis. So far as concerns the histological classification of uterine cancers and the relation between cell type and radiosensitivity, the findings are stated to be based mostly on the work of Dr. Helen Chambers. After a definition of the four anatomical stages of carcinoma of the cervix, the relative values of surgical and radiation treatment are discussed, and a statistical review compares the results obtained from surgery, from surgery and radiotherapy combined, and from radiotherapy alone.

An account is given of the general principles of radiation therapy, the factors conditioning such treatment, and the clinical and histological effects produced. Separate chapters are devoted to clinical technic and to the use of intracavitary radiation. In the latter are described the method and results of dosage calculations, the details of technic employed at the Marie Curie Hospital, the spatial distribution of radiation, and the clinical importance of physical data; acknowledgments are made to Professor W. V. Mayneord for revision of this section. Other chapters deal with the closer evaluation of the results of radium treatment, with cancer of the cervix in pregnancy, and with multiple neoplasms associated with cancer of the uterus. The scope of the work is considerably wider than is indicated by the title since other subjects, such as endometriosis, cancer of the vagina and the vulva, and the treatment of uterine hemorrhage arising from causes other than cancer, are also covered. The book is provided with subject and author indexes and a bibliography.

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